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INSTRUCTIONS TO AUTHORS

Three numbers of the Journal are published every year, in April, August and December respectively and contributions for publication should be sent to the Editor not later than February 1, June 1, and October 1 respectively.

Contributors are requested to be clear and concise. Manuscripts should not exceed 8,000 words and should be in a final form for the press. Each paper should start with a short summary which should be an abstract of the whole paper, complete and clear in itself, and not over 3 per cent. of the length of the paper. The introduction and reviews of literature should be restricted to closely pertinent papers.

The manuscript should be typewritten on one side of the paper only, with wide margins and be double spaced throughout including titles, footnotes, literature citations and legends. Symbols, formulae and equations must be written clearly and with great care. Scientific names of genera and species are printed in italics and should be underlined in the typescript. Too many tables, graphs, etc. should be avoided. Each table should be typed on a separate sheet with its proper position marked in the text in pencil.

Literature citations: All references to literature cited in the text should be presented together at the end of the paper in alphabetical order of authors' names. Each reference should be given in a standard form as follows: (1) name(s), followed by initial(s), of author(s); (2) year of publication in brackets; (3) full title of paper; (4) title of journal, abbreviated according to *World List of Scientific Periodicals*, 1952, and underlined; (5) volume number in Arabic numerals, underlined with two lines to indicate bold type; (6) page numbers without the prefix, p. When books are mentioned in the references, the order should be: name of author(s), initial(s), year in brackets, title of the book, which should be underlined, volume number, edition, page, followed by place of publication and name of publisher. Where a reference has not been seen in original, it should be indicated by an asterisk and the name of the abstracting journal or other source should be mentioned in brackets. If the title is in a language other than English, the diacretic signs, etc., should be precisely given as in the original.

Examples:

Text: (Patel, 1948); but, e.g., 'Patel (1948) showed that . . . .' . For two authors, write as, e.g., Khanna & Sharma (1947), using the ampersand (&). If there are more than two authors, all names should be given when cited for the first time and thereafter the first name only, adding *et al.*

References:

Raman, C. V. (1949) The theory of the Christiansen experiment. *Proc. Indian Acad. Sci.*, A, 29: 381-90.

Sahni, B. (1936a) Wegener's theory of continental drift in the light of Palaeobotanical evidence. *J. Indian bot. Soc.*, 15: 31-32.

Sahni, B. (1936b) The Karewas of Kashmir. *Curr. Sci.*, 5: 10-16.

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Communications should be addressed to Professor T. S. Sadasivan, Editor, Journal of the Madras University (Section B), University Botany Laboratory, Madras-5, India.

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J. Madras Univ., B, XXVI, No. 3, pages 423-425, 1956.

Note on the Differences between Copepods of the Genus *Lernaea* and other *Lernaeids*

BY

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(Received for publication, August 24, 1956)

From the earliest times the genus *Lernaea* in the family Lernaeidae has been "the final dumping ground for everything that was bizarre in form and that could not be located elsewhere" (Wilson 1917b, p. 34). Several of such have been removed and correctly placed in other families and Wilson while revising the family removed many more. What was left he regrouped into four new sub-families of which Lernaeinae contained the three genera *Lernaea* Linneaus *Leptochelus* Brian and *Therodames* Kroyer. Of these the last two are monotypic little known genera and it is not surprising that the genus *Lernaea*, and therefore the subfamily Lernaeinae, present differences from the other genera and subfamilies of Lernaeids. Brühl (1860) advocated the separation of the genus *Lernaea* to form a distinct family but Wilson considered the differences as of "degree rather than in kind" and therefore not sufficient to warrant the creation of a distinct family. However the present author who had studied the anatomy and development of *Lernaea chackoensis* as well as the anatomy of lernaeids belonging to the genera, *Peniculus, Pennella, Lernaeenicus, Cardiodectes* etc., felt that it will be useful to present the differences so as to enable a future correct appraisal of the relationships of the genus.

In his discussion of differences, Wilson has omitted several features or not treated them fully. 1. The foremost difference is that of habit. All the species of *Lernaea* are freshwater forms throughout their life, arguing a physiological and anatomical specialisation not necessary in all other lernaeids which are marine. 2. The life histories of the forms studied show that the copepodids of *Lernaea* are free swimming, have well-developed natatory limbs and can change their hosts at will. But the copepodids of other

lernaeids have a frontal filament like several caligoids and cannot change their hosts. The copepodids of *Lernaea* sp, have well developed antennae, antennules as well as the maxillipedes. These which are setose and spiny serve to secure the temporary attachment. Of the five pairs of thoracic legs present, at least the first four are well developed and the larvae are very swift swimmers. The adults are attached by four arms arranged crosswise (though subsequent elaboration may mask this original four-rayed pattern) and developed from just behind the cephalic region very early in the process of metamorphosis. In species belonging to genera like *Pennella, Lernaeenicus, Cardiodectes* etc., the attachment is by a number of processes springing from the head without any reference to a basic cruciform plan. 3. Wilson has observed in *Lernaea* that "the body is formed by a lengthening of all the thoracic segments, instead of only the fifth and sixth". But he considers that this feature is to be found in a less marked scale in *Peniculus*. A reference to figures on pages 78 and 81 (in No. 6 of literature cited below) will show that that the head and first four thoracic segments form 34 units out of the 57 units of the total length of the young parasite while the fifth and sixth segments and abdomen form only 23 units. When this grows into the adult, there is practically no abdomen which is reduced to a small swelling, the fifth and sixth segments form 80 units against the 12 units of head and first four throcic segments. Almost the 8/9 of the increase in the length of the adult is through the genital segment. This is clearly different from the uniform elongation of all the thoracic segments noted in *Lernaea* in which the genital segment forms only 17% of the total length (vide figure 1 on p. 144 and table on p. 154 of No. 1 and 2 of literature cited as well as fig. 23 of No. 4 of literature cited).

4. The differences in the internal anatomy especially in the genital system are unmistakable. Even Wilson does not attempt to reconcile the fact that egg filaments are formed in the ovary and several rows of eggs are contained in the external sac in *Lernaea* sp, whereas in other lernaeids eggs are formed singly and are arranged in uniseriate egg-strings. To these must be added the fact that there is no separate cement gland in *Lernaea* sp, the posterior half of the oviduct acts in this capacity, whereas in other lernaeids like *Peniculus* and *Cardiodectes* the cement gland is a separate and distinct structure long and cylindrical lying close and parallel to the oviduct. In these the seminal reservoirs, cement

glands and the oviducts are straight and not coiled and run from one end to the other of the long genital segment. In *Lernaea* sp., the reservoirs are small bags lying in the abdominal region and the oviducts are long and coiled tubes contained in the shorter genital region.

5. The nature and disposition of the mouth appendages in *Lernaea* sp, differ from those of other lernaeids. There are two pairs of maxillae in *Lernaea* and the maxillipedes are characteristic. They are columnar, stout and two-jointed. They are forwardly directed and bear five spines. The labrum and labium are separate and distinct, chitinous plates. The mandibles and the maxillae are disposed on either side of the mouth which is slightly protrusible. In the other lernaeids there is a well developed chitinous proboscis or mouth-tube formed by the fusion of the labrum and labium. This tube encloses the mandibles and the maxillae which are much reduced in size. The rim of the tube is supported by chitinous rings and fringed by hairs and spines and can be buried deep into the tissues of the host. The maxillipedes are usually reduced and lie behind the mouth-tube. They end in a single claw recalling the maxillipedes of Caligidae. These differences are very pronounced in the young females (vide *Lernaea*, fig. 25 and *Lernaeenicus* sp, fig. 24 of No. 2 and 4 of literatures cited below).

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J. Madras Univ., B, XXVI, No. 3, pages 427-449, 1956.

On a Collection of Plants from Courtallam

BY

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(Received for publication, August 27, 1956)

ABSTRACT

This contribution records 206 species of flowering plants occurring in Courtallam, Tirunelveli District. A short account of the vegetation of the area precedes the enumeration of plants.

Introduction

The plants enumerated in this article were collected during two botanical excursions, the itineraries of which were as follows:

Excursion I. 27-10-1954 — Chittaruvi area.
28-10-1954 — Shembagadevi arivi area.
29-10-1954 — Terkumalai Estate.
30-10-1954 — Aindalai arivi area.

Excursion II. 18-9-1955 — Aindalai arivi area.
19-9-1955 — Shembagadevi arivi area and Tenarivi.
20-9-1955 — Terkumalai Estate.
21-9-1955 — Tiger Falls area.

In addition to the actual localities specified above, collections were also made along the road or forest paths leading to the respective areas from Courtallam town proper, which formed the starting point. (Fig. 1).

All the specimens collected during the above excursions have been deposited in the Herbarium of the Presidency College, Madras.

The earliest and significant collection in the Courtallam hill ranges was made by Robert Wight. He published a preliminary account of the general botanical features of the area (1835-36). This account is mainly concerned with the description of medicinal and therapeutic properties of a few plants from his collection. We also learn from this account that he visited the area twice

during July-August; that he collected on the northern slopes on one occasion, and along the south-western slope on the other; that

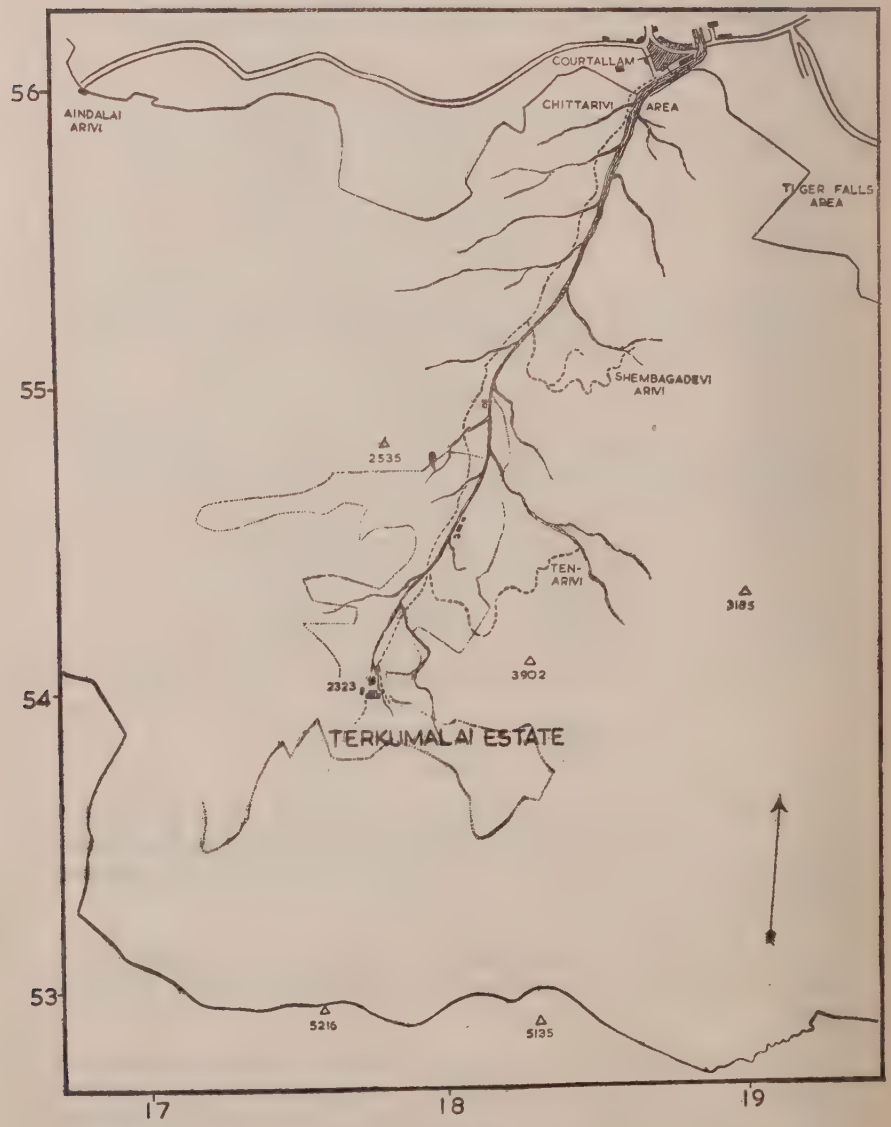

Fig. 1

he collected nearly 600 species, a very large number of which proved to be novelties to science.

A comparison of the list of plants collected by Wight on these two occasions with our own indicates that the areas and routes perambulated by the two parties are different. Wight appears to have concentrated largely in localities of higher altitudes and heavier rain fall (south-western slopes), whereas our routes are primarily confined to the lower altitudes receiving lesser rain. Therefore, we feel that the present enumeration is a supplement to Wight's record.

The flora of Courtallam presents features of interest from the point of view of ecology as well as of floristics. The vegetation, on the whole, is of the semi-evergreen deciduous type at higher altitudes, and of a low scrub at lower levels. This situation is to be attributed, perhaps, to the topographic position of the hill range and the operation of the consequent climatic factors. Courtallam, being in the neighbourhood of the Western Ghats, experiences a heavy down pour of rain contributed by the south-west monsoon, (May-July) and in the remaining part of the year there is a spell of relatively high temperature causing aridity in open areas except in the sholas. A high percentage of relative humidity prevails in those places where there are perennial torrents, small meandering streams and rivulets. In conformity with these factors, the vegetation exhibits a variety of characteristic growth forms.

The north-east part of the hill is situated far away from the Western Ghats, and is subjected to a relatively scanty precipitation. It is these regions, particularly the foot-hill areas, that are verdured with scrub. The most important constituents of this part of the vegetation are: *Plecospermum spinosum, Streblus asper, Zizyphus oenoplia, Z. xylocarpa, Z. nummularia, Dichrostachys cinerea, Pithecolobium dulce, Prosopis spicigera, Securinega virosa, Gymnosporia wallichiana, Lantana* species, *Blachia calycina,* and climbers like *Ichnocarpus frutescens, Pergularia extensa, Cissampelos pariera, Derris scandens,* etc.

As one proceeds interior, towards Terkumalai Estate, there is a conspicuous change of vegetation. Trees, mostly deciduous like *Dalbergia, Anogeissus, Terminalia, Tectona* (cultivated), *Sterculia, Grewia, Mallotus, Macaranga, Ficus* species, etc., begin to appear as dominant constituents. Underneath the tall trees are encountered small evergreen trees and shrubs like *Goniothalamus, Mitrephora, Uvaria, Polyalthia, Psychotria, Randia, Ixora,* etc. The undergrowth vegetation comprises of herbaceous annuals and perennials like

Ophiorrhiza, Pilea, Pouzolzia, Andrographis, Impatiens species, *Biophytum,* etc., in addition to grasses and sedges.

Associated with rock clefts and crevices are found *Oxalis, Sonerila, Hydrocotyle, Pouzolzia,* etc.

The north-eastern part of the hill facing the plains, on account of scanty rain fall, wind action, and exposure, supports a small area of grass land vegetation, in which species of *Panicum, Paspalidium,* etc., are intermingled with *Sopubia, Drosera,* etc. On either side of water courses flowing in the grass land, small semi-aquatic species like *Lindernia, Utricularia, Kyllinga, Fimbristylis, Carex, Scleria, Eriocaulon,* etc., constitute characteristic components. *Leucaena, Prosopis, Grewia, Phyllanthus, Breynia,* etc., demarcate the boundaries of the grass land.

Enumeration

Acanthaceae

1. *Andrographis paniculata* Nees
   Erect herb, sometimes slightly woody towards the base. Flowers pink. Rare. (Terkumalai).
2. *Asteracantha longifolia* Nees
   Hispid thorny herb found in marshes; flowers pale blue to purple. (Aindalai arivi)
3. *Barleria involucrata* Nees
   Unarmed shrub, 1-2 m. tall. Flowers in terminal and axillary racemes. Flowers blue. (Terkumalai).
4. *Elytraria acaulis* Lindau
   Stemless herb with radical leaves. Inflorescence a long cylindrical scape. Found in isolated shady places. Scarce. (Terkumalai).
5. *Justicia diffusa* Willd.
   Slender herb, sometimes slightly suffrutescent. Common. (Shembagadevi).
6. *Justicia glauca* Rottl.
   Herb, young branches pubescent. Corolla white with purple spot. Common. (Chittaruvi).
7. *Ruellia tuberosa* Linn.
   Undershrub with large flowers, deep blue in colour. (Terkumalai).

8. *Rungia parviflora* Nees
A small herb with blue flowers in spikes. Common in shady places. (Aindalai arivi).

Aizoaceae

9. *Mollugo pentaphylla* Linn.
Herb, common on hard soil at low elevations. (Shembagadevi).

10. *Achyranthes bidentata* Bl.
Erect herb; leaves variable in size and shape. Common. (Terkumalai).

11. *Aerva lanata* Juss.
Many-branched undershrub with small whitish axillary spikes. Common. (Aindalai arivi).

12. *Amarantus spinosus* Linn.
Common weed around tenements. (Aindalai arivi).

13. *Celosia argentea* Linn.
Herb, with long silvery spikes, pink at first and changing to white later. Common in cultivated fields. (Aindalai arivi).

14. *Digera muricata* (Linn.) Mart.
Slender herb with prostrate branches common on waste land. (Shembagadevi).

15. *Gomphrena decumbens* Jacq.
Introduced weed commonly found on waste land. (Aindalai arivi).

16. *Psilotrichum nudum* Miq.
This species has been included on the authority of a collection by P. F. Fyson *5062*, 21-11-1916. (Terkumalai).

Amaryllidaceae

17. *Curculigo orchioides* Gaertn.
Perennial stemless herb with cylindrical tapering root stock; very variable in leaf size and shape. Common. (Shembagadevi).

Annonaceae

18. *Goniothalamus thawaitesii* Hook. f. & Thoms.
Small tree, 4-5 m. tall. Flowers solitary, greenish yellow. Scarce. (Shembagadevi).

19. *Mitrephora heyneana* Thw.
Small tree, 4-5 m. tall; flowers yellow; rare. (Shembagadevi).

20. *Polyalthia cerasoides* Hook. f. & Thoms.
Small evergreen tree; flowers green. Rather rare (Shembagadevi).

21. *Uvaria zeylanica* Linn.
Straggling shrub, leaves glabrous. Rare (Terkumalai).

Apocynaceae

22. *Alstonia venenata* R.Br.
Shrub, up to about 3 m. tall; flowers white. Common along forest paths. (Terkumalai).

23. *Carissa carandas* Linn.
Tall shrub, 1-2 m. high. Fruit a red berry, edible. Common in scrub. (Tiger Falls area).

24. *Ichnocarpus frutescens* R.Br.
Much-branched climber; younger parts rusty-villous. Flowers greenish white. Common in scrub. (Aindalai arivi).

Aristolochiaceae

25. *Aristolochia indica* Linn.
Perennial twiner common on hedges and bushes along roadsides. Leaves very variable in size and shape. (At lower levels and Terkumalai).

Asclepiadaceae

26. *Brachylepis nervosa* Wt. & Arn.
Wiry climber with rather thick elliptic leaves. Flowers in dichotomous cymes. (Chittaruvi).

27. *Cynanchum pauciflorum* R.Br.
Climber. Flowers greenish. Stipule-like emergences from leaf axils. Found at an altitude of 650 m. This species has been included on the authority of a collection by P. F. Fyson *5070,* 21-12-1916.

28. *Gymnema elegans* Wt. & Arn.
Twiner; flowers white. Common on bushes along roadsides and in scrub. This species has been included on the authority of a collection by P. F. Fyson *5087,* 20-11-1916.

29. *Gymnema sylvestre* R.Br.
Large twiner; flowers yellowish. Common on bushes in scrub. (Chittaruvi).

30. *Hemidesmus indicus* R.Br.
Wiry climbing shrub; flowers greenish yellow; roots have characteristic odour. Common on bushes in scrub. (Aindalai arivi).

31. *Tylophora indica* (Burm. f.) Merr. Twiner with leaves rather variable in shape and size; flowers greenish yellow; common along roadsides and scrub. (Tiger Falls).

Begoniaceae

32. *Begonia malabarica* Lamk.
Succulent shrub with pinkish red flowers and large winged capsules. In shady places. This species have been included on the authority of a collection by P. F. Fyson, *5056*, 20-12-1916.

Bixaceae

33. *Flacourtia sepiaria* Roxb.
Armed shrub, 1-2 m. tall. Flowers borne on the thorns. Common in scrub jungles. (Shembagadevi and Chittaruvi).

34. *Hydnocarpus alpina* Wt.
Large evergreen tree; fruit a many-seeded woody berry. Only one tree was met with. (Terkumalai).

Boraginaceae

35. *Ehretia microphylla* Lamk.
Shrub, 1 — 1·5 m. tall; leaves scabrid; flowers white. Common in scrub. (Shembagadevi).

36. *Ehretia ovalifolia* Wt.
Small tree, about 4 m. tall; leaves glabrous; flowers white. Less common. (Shembagadevi).

37. *Heliotropium scabrum* Retz.
Perennial woody herb, common on waste ground. Leaves scabrid. Flowers white. (Chittaruvi).

Caesalpiniaceae

38. *Cassia occidentalis* Linn.
Small shrub, rather diffuse; flowers yellow. Open hard soil, common at lower elevation. (Shembagadevi).

Cannaceae

39. *Canna indica* Linn.
Perennial herb growing in clumps; flowers light yellow. All ovules mature into viable seeds. In moist open places. (Terkumalai).

Capparidaceae

40. *Capparis diversifolia* Wt. & Arn.
Thorny shrub; branches zig-zag. Inflorescence an umbel. (Shembagadevi).

41. *Capparis olacifolia* Hook. f. & Thoms.
Armed shrub. Not common. (Terkumalai).

Caryophyllaceae

42. *Cerastium indicum* Wt. & Arn.
Weak-stemmed herb, somewhat spreading on the ground. Inflorescence, a much-branched dichotomous cyme. Flowers small and greenish. Common in wet places. (Terkumalai).

43. *Polycarpaea corymbosa* Lam.
Common weed found in waste places; also in cultivated fields at low elevations. (Chittaruvi).

Celastraceae

44. *Gymnosporia emarginata* Lawson
Shrub with long thorns often bearing leaves and flowers. Common in open scrub. (Chittaruvi).

45. *Gymnosporia wallichiana* Spreng.
Shrub, branches zig-zag. Thorns often bear leaves and flowers. Common in open scrub. This species is included on the authority of a collection by Barber *3414*, 18-7-1901.

Chloranthaceae

46. *Sarcandra irvingbaileyi* Swamy
We have not seen this species in the areas we visited. But we are including this on the authority of a herbarium specimen collected in August 1899; the sheet bears the initials of the collector, K.H.R. No number is given.

Combretaceae

47. *Anogeissus latifolia* Wall.
Deciduous tree, bark grey and smooth; undersurface of leaves ashy white, Common. (Sherbagadevi, Chittaruvi).

48. *Terminalia paniculata* Roth.
Large deciduous tree with characteristic grooved fruits. Occasional. (Chittaruvi, Aindalai arivi).

Commelinaceae

49. *Aneilema montana* Wt.
Annual herb; flowers in lax terminal panicles. Flowers blue. Common on exposed wet slopes. (Terkumalai).

50. *Aneilema nudiflorum* R.Br.
Annual procumbent herb, often rooting at the nodes. Flowers blue in terminal panicles consisting of cymose flower clusters. Common. (Shembagadevi).

51. *Commelina clavata* Clarke
Herb, flowers lilac. In moist places. Rather scarce. (Shembagadevi).

52. *Commelina salicifolia* Roxb.
Slender herb with creeping stems. Flowers deep blue. In wet places. Common. (Shembagadevi).

53. *Cyanotis axillaris* Röem. & Sch.
Prostrate herb, fairly common. Flowers blue or pink. (Shembagadevi).

54. *Cyanotis villosa* Schult. f.
Erect herb, stems and leaves often dark purple; flowers blue. Common at foot hills. (Shembagadevi).

Compositae

55. *Acanthospermum hispidum* DC.
Annual herb. Achenes with a pair of long horn-like spines at the apex. Perhaps introduced and run wild. (Shembagadevi).

56. *Bidens pilosa* Linn.
Perennial herb; leaves variable in size and shape. Achenes provided with a pair of barbed pappus. Common. (Terkumalai).

57. *Conyza ambigua* DC.
Erect soft villous herb; flowers yellow. Fairly common but not indigenous. (Terkumalai).

58. *Erigeron mucronatum* DC.
Annual herb, run wild. (Terkumalai).

59. *Gynura nitida* DC.
Shrub with orange coloured florets. Not very common. (Aindalai arivi).

60. *Notonia grandiflora* DC.
Succulent shrub, reaching nearly 2 m. in height. Florets yellowish green. (This specimen conforms to the description given by Gamble in the Flora of the Presidency of Madras, p. 717 in regard to the plants occurring in Courtallam.).

Convolvulaceae

61. *Evolvulus alsinoides* Linn.
Perennial spreading herb, with a woody root stock; branches wiry and radiating; flowers light blue. Common at lower elevations (Aindalai arivi).

62. *Lettsomia aggregata* Roxb.
Climbing shrub, with pink flowers. Fruit red. Frequent on shrubs and trees along roadsides and scrub. (Aindalai arivi).

Crassulaceae

63. *Kalanchoe floribunda* Wt. & Arn.
Succulent herb; on rocks covered with dripping water. Flowers yellow. Not found elsewhere. (Tenarivi).

Cucurbitaceae

64. *Melothria mucronata* Cogn.
Slender climbers; common on hedges and bushes. (Terkumalai).

Cyperaceae

65. *Fimbristylis dichotoma* Vahl.
Common in grass lands and waste grounds. (Terkumalai).

66. *Hackelochloa granularis* O. Ktz.
Annual erect herb, much branched. Common in drier tracts. (Aindalai arivi).

67. *Kyllinga melanosperma* Nees
Rhizomatous herbs, stems 2 to many. Common. (Shembagadevi).

68. *Kyllinga squamulata* Vahl.
Common. (Shembagadevi).

69. *Scleria lithosperma* Sw.
Erect herb, common amidst grasses. (Terkumalai).

Dioscoreaceae

70. *Dioscorea oppositifolia* Linn.
Twiner, stem almost glabrous. Tuber underground and very long, edible. Common at lower elevations. (Shembagadevi).

71. *Dioscorea pentaphylla* Linn.
Tuberous climbing herb, rather variable in vegetative characters. Prickly towards the base, younger parts rusty-hairy. (Shembagadevi).

Droseraceae

72. *Drosera burmanni* Vahl.
Stemless herb, insectivorous. Leaves spathulate and rosetted at the base. Leaves green when young becoming deep red with age. On marshy grass land. (Tiger Falls).

Euphorbiaceae

73. *Acalypha paniculata* Miq.
Small undershrub with paniculate inflorescence. Fairly common. (Aindalai arivi).

74. *Blachia calycina* Benth.
Tall shrub, about 3 m. high. Calyx persistent in fruit. Common in exposed belts. (Chittaruvi, Tiger Falls).

75. *Croton klotzschianus* Thw.
Shrub or small tree. Young leaves stellate-hairy. Quite common at about 650 m. altitude. (Aindalai arivi and Chittaruvi).

76. *Givotia rottleriformis* Griff.
Tree, 4-6 m. tall; underside of leaves densely white tomentose. Bark brown. Drupe about 1" long. Rather scarce. (Shembagadevi, Terkumalai).

77. *Glochidion ellipticum* Wt.
Tree, with shiny foliage. Rare. (Terkumalai).

78. *Mallotus phillippinensis* Müll. Arg.
Tree, leaves and young branches, rusty-tomentose; bark grey. Quite frequent in deciduous zones. Cultivated near the Forest Rest House. (Chittaruvi).

79. *Melanthesa turbinata* (Koen. ex Roxb.) Wt.
Shrub, 1-1.5 m. tall. Calyx enlarged in fruit. Fruit orange coloured. Common in open scrub. (Terkumalai).

80. *Phyllanthus missionis* Hook. f.
Erect undershrub, found occasionally in the immediate vicinity of water falls. (Aindalai arivi).

81. *Phyllanthus simplex* Retz.
Stiff suffrutescent herb with almost flattened branches. Common. (Chittaruvi).

82. *Phyllanthus urinaria* Linn.
Perennial erect herb; leaves more or less sensitive according to Gamble. Young leaves pinkish. In forest undergrowth. (Shembagadevi).

83. *Reidia floribunda* Wt.
Not collected by us. We have included on the authority of a collection by Barber *3383*, 18-7-1901.

84. *Reidia longiflora* Gamble
Small bush below 1 m. tall. Flowers pink. Common. (Terkumalai).

85. *Securinega leucopyrus* (Willd.) M. Arg.
Large straggling shrub with sharp spinous branches. Fruit white. Common in scrub. (Aindalai arivi).

86. *Securinega virosa* (Roxb.) Pax. & Hoffm.
Unarmed large shrub. Foliage variable in size and shape. Along roadsides. Not very common. (Shembagadevi).

87. *Tragia involucrata* Linn.
Perennial weak-stemmed undershrub, usually straggling with stinging hairs. In waste places. (Aindalai arivi).

Geraniaceae

88. *Biophytum candolleanum* Wt.
Perennial herb, stem unbranched, of variable height and thickness. Common in undergrowth. Flowers yellowish-pink. (Chittaruvi).

89. *Impatiens fruticosa* DC.
Soft erect shrub; flowers pink; in shady places. Not common. (Terkumalai).

90. *Oxalis corniculata* Linn.
Herbaceous creeper with yellow flowers in umbels. Common on forest floor and also along roadsides. (Shembagadevi and Aindalai arivi).

Gesneriaceae

91. *Didymocarpus rottleriana* Wall.
Stemless herb, found in the crevices of rocks in the immediate vicinity of water falls. Flowers purple. (Shembagadevi).

92. *Klugia notoniana* A.DC.
Succulent annual herb; leaves asymmetric with many prominent curved nerves. Flowers bright blue. Found in damp sholas. (Chittaruvi).

Gramineae

93. *Alloteropsis cimicina* Stapf
Perennial erect herb, common on open ground. (Aindalai arivi).

94. *Amphilophis pertusa* Stapf.
Perennial herb, on open ground. Rather scarce. (Terkumalai).

95. *Chloris barbata* Sw.
Perennial erect herb, very common. (Aindalai arivi).

96. *Cymbopogon polyneuros* Stapf.
On open ground, common. (Terkumalai).

97. *Eragrostis tremula* Hochst.
Slender herb, common. (Shembagadevi).

98. *Eragrostis willdenoviana* Nees
Perennial erect slender herb. Common. (Shembagadevi).

99. *Paspalidium flavidum* A. Camus
Perennial herbs; inflorescence a raceme of spikes that are sessile and distantly placed. Common. (Terkumalai).

100. *Sporobolus diander* Beauw.
Perennial erect herb. Common over a wide area. (Terkumalai).

Hippocrateaceae

101. *Salacia ? reticulata* Wt.
Woody liane; fruit tuberculate. Our specimens being incomplete the specific determination is provisional. (Shembagadevi).

Icacinaceae

102. *Nothapodytes foetida* (Wight) Sleumer,
Tree, 10 — 15 m. tall, with dark green foliage.

Recognition of the generic name used above is based up on the conclusions of Howard (Jour. Arnold Arb. 23, p. 70, 1942). (Tenarivi).

Labiatae

103. *Anisomeles malabarica* R.Br.
Shrub, about 2 m. tall; densely white woolly. Flowers purple. Along roadsides bordering cultivated fields. Scarce. (Aindalai arivi).

104. *Leucas aspera* Spreng.
Perennial herb, common on waste and cultivated lands. On the plains and foot of the hills. (Shembagadevi and Chittaruvi).

105. *Ocimum adscendens* Willd.
Erect herb, flowers pale crimson. Fairly common around the water falls. (Shembagadevi).

106. *Plectranthus coesta* Buch.-Ham.
Tall herb; leaves white tomentose beneath; flowers lavander-blue. On the fringes of sholas. (Terkumalai).

107. *Scutellaria violacea* Heyne
Herb, rather scarce. (Terkumalai).

Lentibulariaceae

108. *Utricularia striatula* Sm.
Terrestrial herb; stems rooted in marsh. Leaves persistent and bear many small bladders. On wet rocks. (Shembagadevi).

109. *Utricularia stricticaulis* Stapf
Stem thick, flowers blue. Along water course. (Tiger Falls).

Liliaceae

110. *Chlorophytum malabaricum* Baker
Perennial herb, with tuber-like fleshy fascicled roots. Rare. (Terkumalai).

111. *Smilax wightii* A. DC.
Climbing shrub, generally rare. (Shembagadevi).

112. *Smilax zeylanica* Linn.
Climbing shrub. Along forest paths. Scarce. (Terkumalai).

Linaceae

113. *Erythroxylon monogynum* Roxb.
Small tree, 2 — 3 m. tall. Bark rough, dark brown; heart wood very hard and scented. Flowers dull white, fruit red. (Chittaruvi, Aindalai arivi).

114. *Hugonia mystax* Linn.
Rambling shrub; young twigs tomentose; two axillary spirally coiled hooks at nodes; flowers yellow, conspicuous. Bark yellowish and corky. Common in scrub. (Tiger Falls).

Loranthaceae

115. *Dendropthoe falcata* (Linn. f.) Etting.
Parasitic shrub; inflorescence a raceme; flowers scarlet, orange or pink. (Aindalai arivi).

116. *Viscum articulatum* Burm.
Leafless stem parasite found on the branches of *Dalbergia* species. (Aindalai arivi).

Lythraceae

117. *Lagerstroemia lanceolata* Wall.
Large deciduous tree; flowers white, small. Bark smooth, whitish, peeling off in papery flakes. Rather scarce.

This specimen has been included on the authority of a collection by Barber *3303*, 29-6-1901.

Malpighiaceae

118. *Tristellateia australis* A. Rich.
Flowers yellow. Cultivated. (Terkumalai).

Malvaceae

119. *Sida acuta* Burm.
Almost a weed in open forests and plains. (Aindalai arivi).

120. *Sida rhomboidea* Roxb.
Small undershrub; leaves white tomentose-beneath. Commonly associated with open forest floor. Leaves appear to vary from more typical specimens of the species. (Terkumalai).

Meliaceae

121. *Aglaia bourdillonii* Gamble
Small tree, 5 m. tall. Flowers in rather dense panicles. Not common. (Shembagadevi).

122. *Cipadessa baccifera* Miq.
Bushy shrub; drupes reddish orange. Common in open jungles. (Terkumalai).

Menispermaceae

123. *Cissampelos pareira* Linn.
White tomentose climber with cordate leaves. Bracts of female racemes conspicuous; fruit a hirsute scarlet drupe. Common along roadsides on hedges and in scrub. (Terkumalai and Aindalai arivi).

124 *Diploclisia glaucescens* (Bl.) Diels
Large climber with large rotund cordate leaves. Inflorescences in drooping panicles arising from old stems. Flowers yellow. Very rare. (Aindalai arivi).

125 *Tiliacora acuminata* Miers
Climbing shrub, leaves glabrous and shiny; flowers yellow; drupe red. Fairly common. (Chittaruvi).

Mimosaceae

126. *Dichrostachys cinerea* Wt. & Arn.
Thorny shrub. Inflorescences partly pink and partly yellow. Occasional. (Shembagadevi).

Moraceae

127. *Ficus tomentosa* Roxb.
Tree, with aerial roots; bark greenish white; leaves grey tomentose beneath and a gland at the base of the midrib. (Shembagadevi).

128. *Plecospermum spinosum* Tréc.
Large rambling shrub with milky latex; very conspicuous stout, long straight thorns. In open scrub. (Tiger Falls).

Myristicaceae

129. *Myristica fragrans* Houtt.
Cultivated in the Estate grounds. (Terkumalai).

Myrtaceae

130. *Syzygium jambos* (Linn.) Alston
Small tree; flowers large, creamy white. Occasional. (Shembagadevi).

Ochnaceae

131. *Ochna gamblei King*
Small tree with thick bark; glaucous leaves tufted at the ends of branches; flowers conspicuous and yellow. Occasional. (Chittaruvi).

Oleaceae

132. *Jasminum brevilobum* A.DC.
Large climbing shrub, stem spirally twisted; branches and leaves fulvous tomentose; flowers white. (Shembagadevi).

133. *Linociera zeylanica* Gamble
Small tree. Inflorescence panicled. Occasional. (Chittaruvi).

134. *Nyctanthes arbor-tristis* Linn.
Appears cultivated in the Estate. Recently there have been attempts to include this genus in the Verbenaceae. For detailed information see N. L. Bor, Manual of Indian Forest Botany, p. xiv and literature cited therein. (Terkumalai).

Onagraceae

135. *Ludwigia parviflora* Roxb.
Erect herb, less than 1 m. tall. Flowers small and yellow. Capsule slightly inflated at top. In marsh. (Shembagadevi and Aindalai arivi).

Opiliaceae

136. *Cansjera rheedii Gmel.*
Large climber with occasional spines on the lower parts of stem. Inflorescence in axillary spike. Flowers greenish yellow. Rather scarce. (Shembagadevi).

Orchidaceae

137. *Oberonia zeylanica* Hook. f.
Epiphytic herb with plicate ensiform succulent leaves; Flowers minute, pale yellow in simple raceme. On tree trunks and branches in sholas. (Tenarivi).

Papilionaceae

138. *Atylosia albicans* Benth.
Herbaceous climber with striate stem; venation on leaflets very prominent. Flowers yellow.
This species has been included on the authority of a collection by P. F. Fyson *5046*, 21-12-1916.

139. *Derris scandens* Benth.
Large scandent climber with drooping branches; flowers white on pendant peduncles. Common in open forest. (Tiger Falls).

140. *Indigofera pulchella* Roxb.
Large shrub up to 2 m. tall. Flowers purple. In drier areas. (Shembagadevi).

141. *Tephrosia purpurea* Pers.
Undershrub with pink flowers. Common on road sides. (Aindalai arivi).

Piperaceae

142. *Hackeria subpeltata* Kunth
Soft shrub with characteristic large orbicular deeply cordate leaves; inflorescence erect from leaf axil. (Terkumalai).

143. *Poperomia dindigulensis* Miq.
Succulent herbaceous epiphyte on tree trunks and branches; in moist shady places. Fairly common (Terkumalai).

144. *Piper nigrum* Linn.
Cultivated in gardens. (Aindalai arivi).

145. *Piper trichostachyon* DC.
Woody climber with dark green leaves. Fairly common. (Terkumalai).

Plumbaginaceae

146. *Plumbago zeylanica* Linn.
Rambling sub-scandent perennial herb, with glandular

hairs on the calyx. Run wild. (Tiger Falls and Shembagadevi).

Polygalaceae

147. *Polygala bulbothrix* Dunn.
Woody herb; bulbous hairs present on all parts. Flowers greenish; keel with pinkish laciniate tip. Common component of forest floor. (Terkumalai, Aindalai arivi, and Shembagadevi).

148. *Polygala chinensis* Linn.
Occasionally found at lower elevations. Flowers yellow. (Aindalai arivi).

149. *Polygala javana* DC.
Small branching undershrub. Flowers pink in erect axillary racemes. In open places, rather rare. (Shembagadevi).

150. *Polygala sibérica* Linn.
This species has been included on the authority of a collection made in October 1935; collector's name and exact locality are not given.

Polygonaceae

151. *Polygonum chinense* Linn.
Rambling undershrub found among the bushes. (Terkumalai).

152. *Polygonum glabrum* Willd.
Erect annual herb; flowers pink. Along the margins of a pond. (Aindalai arivi).

Rhamnaceae

166. *Zizyphus oenoplia* Mill.
Large thorny straggler; leaves silky pubescent. Common in open scrub. (Terkumalai and Shembagadevi).

167. *Zizyphus xylopyra* Willd.
Large straggling shrub; Pericarp velvety. Leaves white woolly when young becoming glabrous with age. (Shembagadevi).

Rubiaceae

153. *Anotis monosperma* Benth. & Hook. f.
A much-branched herb occurring on the floor of dense canopied shola vegetation. (Shembagadevi).

154. *Borreria stricta* K. Sch.
Erect scabrid herb with white flowers. Common along road sides. (Chittaruvi).

155. *Gardenia gummifera* Linn. f.
Small tree 3 — 4 m. tall. Young leaves protected by yellow resin. Bark greyish brown. (Shembagadevi and Chittaruvi).

156. *Ixora lanceolaria* Colebr.
Erect shrub; flowers white in lax corymb. In open forest. (Terkumalai).

157. *Morinda umbellata* Linn.
Diffuse climbing shrub with rather membranous leaves. Flowers in many-branched terminal umbels. Not seen elsewhere. (Terkumalai).

158. *Oldenlandia albo-nervea* Gamble
Glabrous undershrub; leaves whitish beneath. Common. (Terkumalai and Shembagadevi).

159. *Ophiorrhiza mungos* Linn.
Herb, flowers white on terminal reflexed dichotomous cymes. Flower buds turn dull vermillion on drying. Common component of undergrowth in sholas. (Shembagadevi and Terkumalai).

160. *Pavetta indica* Linn.
Large shrub; foliage shiny, flowers white. Quite common. (Chittaruvi).

161. *Plectronia parviflora* Bedd.
Thorny shrub; leaves variable in size; bark grey; flowers white. In undergrowth. (Chittaruvi).

162. *Plectronia rheedii* Bedd.
Thorny shrub; flowers greenish white; in forest undergrowth. (Chittaruvi).

163. *Psychotria connata* Wall.
Not collected by us. We are including this species on the authority of a specimen collected by Barbar *3413*, 18-7-1901.

164. *Psychotria nudiflora* Wt. & Arn.
Large glabrous shrub, leaves becoming dark and wrinkled when dry. Fairly common. (Terkumalai).

165. *Randia malabarica* Lamk.
Erect thorny shrub, bark brown; flowers fragrant. Not seen elsewhere. (Terkumalai).

Rutaceae

168. *Atalantia monophylla* Correa
Small thorny tree, about 3 m. tall. Flowers in umbels or corymbs. Fairly common. (Aindalai arivi and Terkumalai).

169. *Glycosmis arborea* (Roxb.) Correa
Shrub, highly variable in habit, Common. (Aindalai arivi and Chittaruvi).

170. *Murraya exotica* Linn.
Large shrub; flowers white, fragrant. Frequently seen on sides of forest paths. (Shembagadevi).

171. *Toddalia asiatica* Lamk.
Thorny shrub, somewhat variable in exomorphic characters. Common in open scrub at lower elevations. (Chittaruvi).

Salvadoraceae

172. *Azima tetracantha* Lamk.
Characteristic straggling armed shrub with two long thorns from each axil. Berries white. Common at lower elevations. Flowers yellowish green.
This species has been included on the authority of a collection by Barber *3286*, 25-6-1901.

Santalaceae

175. *Santalum album* Linn.
Cultivated near the Forest Rest House and elsewhere. (Shembagadevi).

Sapindaceae

174. *Dodonaea viscosa* Linn.
Shrub with shiny foliage. Capsule winged. Abundant in open fields at lower elevations. (Chittaruvi).

175. *Lepisanthes deficiens* Raddl.
Not seen by us; included on the authority of a specimen collected in October, 1930; no other data available.

176. *Nephelium longana* Camb.
Large evergreen tree; young leaves conspicuously red;

bark smooth. Fruit with sharp tubercled pericarp, rusty brown in colour. Aril edible. Not seen elsewhere. (Chittaruvi and Honey Falls).

177. *Sapindus laurifolius* Vahl.
Tree with dense foliage; leaves slightly pubescent beneath. Occasional. (Shembagadevi).

Scrophulariaceae

178. *Limnophila heterophylla* Benth.
Aquatic erect herb with highly dissected submerged leaves and simple aerial leaves. Found in a stagnant pond. (Aindalai arivi).

179. *Lindernia ciliata* (Colsman) Pennell
Stiff erect herb with pink, purple or white flowers. Common in marsh. (Terkumalai).

180. *Sopubia delphinifolia* G. Don
Erect herb, somewhat suffrutescent. Common near water courses. (Tiger Falls and Chittaruvi).

181. *Striga euphrasioides* (Vahl.) Benth.
Erect root parasitic herb, turning black when dry. Common in grass land. (Aindalai arivi).

Solanaceae

182. *Solanum vagum* Heyne.
Glabrous shrub; flowers white. Common near tenements. (Tenarivi).

Sterculiaceae

183. *Sterculia guttata* Roxb.
Large deciduous tree with conspicuously spreading branches. Leaves rusty-stellate tomentose. Less frequent. (Shembagadevi).

184. *Waltheria indica* Linn.
Undershrub, found in waste places, as well as in forest undergrowth at lower elevations. Flowers yellow. (Aindalai arivi).

Tiliaceae

185. *Grewia barberi* Drumm.
Small shrub, occasionally found in scrub in the immediate vicinity of water fall. (Chittaruvi).

186. *Grewia gamblei* Drumm.
Small tree; twigs and underside of leaves softly tomentose. Common. (Terkumalai).

187. *Grewia microcos* Linn.
Erect shrub; readily recognized by paniculate inflorescence and unlobed fruit. Flowers white. (Tenarivi and Shembagadevi).

188. *Grewia orientalis* Linn.
Straggling shrub; fairly common in scrub and semi-deciduous belts. (Aindalai arivi).

Ulmaceae

189. *Trema orientalis* Bl.
Tree; in forest clearings. Leaves whitish beneath. Rather rare. (Aindalai arivi).

Umbelliferae

190. *Centella asiatica* (Linn.) Urb.
Trailing herb, rooting at nodes. Inflorescence clustered in leaf axil; in wet places. (Shembagadevi).

191. *Hydrocotyle javanica* Thumb.
Herbaceous runner, glabrous. In moist shady places. (Terkumalai).

192. *Pimpinella heyneana* Wall.
Erect annual herb. Not seen by us. This species has been included on the authority of a collection by P. F. Fyson, *5064*, 21-12-1916.

Urticaceae

193. *Elatostemma lineolatum* Wt.
Undershrub, with variable foliage and habit. Leaves falcate and longitudinally unequal. In moist shady places rich in humus. (Shembagadevi).

194. *Pilea muscosa* Lindl.
Succulent herb with tiny rotund and spathulate leaves; introduced and run wild in the Estate. (Terkumalai).

195. *Pouzolzia bennettiana* Wt.
Herb, rather rare. (Terkumalai).

196. *Pouzolzia indica* Gaud.
Slender hirsute herb. Common. (Terkumalai and Shembagadevi).

197. *Pouzolzia wightii* Benn.
Robust herb, rather variable. Common. (Terkumalai).

Verbenaceae

198. *Clerodendrum infortunatum* Linn.
Almost of the size of a small tree, about 5 m. tall. Leaves and twigs densely tomentose. Occasional. (Shembagadevi and Aindalali arivi).

199. *Gmelina asiatica* Linn.
Large shrub with brownish white bark, often the branches ending in a point. Flowers yellow. Less common. (Shembagadevi).

200. *Lantana wightiana* Wall.
Woody shrub with white flowers. Cultivated ? (Terkumalai).

201. *Stachytarpheta indica* Vahl.
Tall herb woody below. Flowers blue. Common on forest floor. (Shembagadevi).

202. *Tectona grandis* Linn. f.
Planted (Aindalai arivi).

203. *Vitex altissima* Linn. f.
Fairly a large tree; flowers blueish white in both axillary and terminal panicles. Less common. (Shembagadevi).

Violaceae

204. *Viola serpens* Wall.
Herb, with a woody root stock. Flowers blue. Common at higher elevations and in shady moist places. (Terkumalai).

Vitaceae

205. *Leea sambucina* Willd.
Large shrub with characteristic clasping leaf bases. Not seen at lower levels. (Terkumalai).

Zingiberaceae

206. *Costus speciosus* Sm.
Succulent herb reaching 2-3 m. height. Stem twisted. flowers white. Occasional (Terkumalai).

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Notes on Pelagic Copepoda of the Madras Coast

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ABSTRACT

The occurrence of twenty pelagic copepods, new to the fauna of Madras are reported. The hitherto undescribed male of *Calocalanus plumulosus* (Claus) and the 4th copepodite of *Scolecithrix danae* (*Lubbock*) are described.

In the course of a study of the pelagic copepods of the Madras coast, 112 species were collected and reported in previous papers by the author (Krishnaswamy 1953 a, b & c). Since then 20 more species have been collected and identified. Most of them are being recorded for the first time at Madras. The copepods reported in the present paper were collected from oblique plankton hauls taken with a closing net. Most of these forms were collected at a depth of 5-10 fathoms, off Madras.

CALANOIDA

Family: Calanidae.
Genus: *Nannocalanus* Sars.

Nannocalanus minaor (Claus) forma *major* Sewell.

Sewell, 1929, p. 21, Text-fig. 2, a-d; 1947; p. 14-15; Text-fig. 1 A.

Occurrence: Several specimens of both the sexes were present in the plankton collected in November 1952 (28th).

Distribution: Widely distributed in the Pacific, Atlantic and Indian Oceans.

Undinula darwini var. *caroli* (Giesbrecht).

Calanus caroli Wolfenden, 1905, p. 994, Pl. 97, fig. 11;

Undinula darwini var. *caroli*, Sewell, 1914, p. 198.

Occurrence: Several males of this variety were found in the oblique hauls made off Madras in November and December 1952.

Distribution: Pacific Ocean (Giesbrecht), East Indies (Vervoort), Malay Archipelago (A. Scott), Bay of Bengal (Sewell), Gulf of Manaar (Sewell), Maldive Archipelago (Wolfenden).

Remarks: The male described by Giesbrecht in 1888 as *Calanus caroli* shows certain differences from *Undinula darwini* especially in the structure of the fifth leg. Sewell (1914) as well as Vervoort (1946) consider it to be only a variety of *U. darwini*.

Family: Eucalanidae.
Genus: *Eucalanus* Dana.

Eucalanus pseudoattenuatus Sewell.
Sewell, 1947, pp. 40-42, Text-fig. 7A and Text-fig. 8 A-F.

Occurrence: Several females from the oblique haul taken at 10 fathoms off Madras in 1951.

Distribution: This species was described by Sewell from the Arabian sea and this is the first record of the occurrence of this species outside that area.

Size: 4.35 mm. long.

Genus: *Rhincalanus* Dana.

Rhincalanus cornutus forma *typica* Schmaus.

Schmaus & Lehnhofer, 1927, pp. 259-265; Fig. 1. Sewell, 1947, pp. 48-49.

Occurrence: A single female from plankton collected in the evening on 6-10-55.

This copepod has a very wide distribution on the Indian and Pacific Oceans.

Family: Paracalanidae.
Genus: *Paracalanus* Boeck.

Paracalanus denudatus Sewell.
Sewell, 1929, pp. 66-68, Text-fig. 23.
Farran, 1936, p. 80, Text-fig. 1 b.
Sewell, 1947, p. 51.

Occurrence: A single female from the plankton collected on 6-10-'55.

Distribution: The Barrier Reefs of Australia (Farran), the Andaman and Nicobar Islands, and the Arabian Sea (Sewell).

Family: Pseudocalanidae.
Genus: *Calocalanus* Giesbrecht.

Calocalanus plumulosus (Claus)

Giesbrecht, 1892, p. 176, Pl. 111, fig. 8 Pl. lx; figs. 2-22; pl. xxxvi; figs. 39-42; Sewell, 1929, p. 89.

Wilson, 1932, p. 41, fig. 23.

Syn: *Calocalanus tenuis* Farran, 1926, p. 235, pl. 5, figs. 7-12.

Occurrence: Several females were collected from the plankton collected on 6-10-'55. Associated with it was a single male.

Distribution: Widely distributed in the Pacific, Indian and Atlantic Oceans.

Although the female was described as early as 1863, the male remained unknown. Hence the occurrence of a single male at Madras is of interest. A brief description of the male is given below.

Length: 1.12 mm.

Resembles the female in the general shape of the body. The urosome is five-jointed as in *C. pavo*. The caudal rami are parallel with the body. The antennule is 25-jointed, reaching upto the end of metasome.

The second joint of the antennule carries two stout plumose setae at its outer distal corner. The mouth parts and the first four pairs of legs as in the female.

The fifth leg is uniramous and asymmetrical as in *C. pavo*. The right fifth leg is 3-jointed, the terminal joint carrying a short spine and two setae. The left fifth leg is slightly shorter than the right one and its distal joint carries two setae apically (Fig. 1).

The females examined at Madras closely agreed with the description of *C. tenuis* Farran, especially in the structure of the fifth leg.

Calocalanus contractus Farran.

Farran, 1926, p. 234, Pl. 5, Figs. 1-4.

Sewell, 1929, p. 89-90, Text-fig. 35, a-d.

Occurrence: A single female from the oblique haul made on 18-12-1952.

Distribution: Indian ocean (Sewell), Bay of Biscay (Farran).

Genus: *Clausocalanus* Giesbrecht.

Clausocalanus farrani Sewell.

Sewell, 1929, p. 94, Text-fig. 38, a-f.

Farran, 1936, p. 81.

Sewell, 1947, p. 55.

Occurrence: Two females from the plankton collected in June 1955.

Distribution: The Great Barrier Reefs of Australia (Farran), the coast of S. Burma, and the Arabian Sea (Sewell).

Family: Pseudodiaptomidae.
Genus: *Schmackeria* Poppe and Richard.

Schmackeria tollingarae (Sewell).

Pseudodiaptomous tollingeri Sewell, 1919, pp. 2-5, pl. 10. Fig. 8. Sewell, 1924, p. 787, Pl. xlv, Fig. 3; 1932, p. 241.

Schmackeria tollingeri Marsh, 1934, pp. 48-49, pl. 23, Fig. 2.

Pseudodiaptomous tollingarae Brehm, 1954, pp. 309-312, Fig. 72-78.

Occurrence: Numerous males and females from the plankton collected at Coum and Adyar, (Dec. 1950) at Madras.

Distribution: At present known from the Chilka Lake, the Salt Canals (Sewell), Pondicherry lagoon (Brehm) and Madras (Present record).

The female:
Length: 1.35 mm long.

The metasome is pointed anteriorly, more or less elliptical in outline, longer than broad, the length and breadth being in the ratio of 53:23. The posterior corners are pointed and carry teeth The metasome segments have the following proportional width; 30:5:7:8. Further, the metasome is longer than the urosome, the two being in the proportion of 50:38. Furcal rami are longer than broad, and each ramus carries one lateral and four apical setae, the third seta from the inner side being lance-shaped. The antennule is 21-jointed. The second joint of the antennule is indistinctly divided.

In the first leg, the basals$_2$ is armed with teeth towards the outer side. The first joint of the exopod has an outer spine and an inner seta, the second an inner seta only and the third two short outer spines, a long apical spine and three setae. The endopod is slightly shorter than the exopod. The first and the second joints carry an inner seta each and the terminal joint one outer, two apical and two inner setae. In the second leg, the basal$_2$ carries a plumose spine on the inner side. The first and the second joints of the exopod carry an outer spine and an inner seta and the ter-

minal joint which slightly longer than the previous joints has two outer spines, a long spine apically and four inner setae. The outer margin of the endopod is hirsute. The first joint carries an inner seta, the second joint two inner setae, while, the terminal joint carries two outer, two inner and two apical setae. The fifth leg is uniramous and three-jointed. The second joint is produced into a blunt process on its inner margin as in *S. smithi* and the terminal joint carries three plumose spines (Fig. 2).

The male is slightly smaller than the female measuring 1.1 mm. The caudal setae are uniform and the spatulate third seta is replaced by normal one. The right antennule is 21-jointed. It bears a finger-like process on the outermargin of the 8th segment and a broad spine on the 15th segment. The second joint of the right fifth leg is very swollen. On the left side it bears a process towards the inner side (Fig. 3).

Family: Scolecithricidae.
Genus: *Scolecithrix* Brady.

Scolecithrix danae (Lubbock).
Rose, 1942, pp. 118-121, Figs. 1-7;.
Chiba, Tsuruta and Maeda, 1955, pp. 206-207, Fig. 6.

Occurrence: Several specimens of both the sexes from the plankton collected in December 1952. Juveniles in the third copepodite stage were collected in the plankton haul made on 6-10-55.

Distribution: Widely distributed in the Pacific, Indian and Atlantic oceans.

Copepodite, IVth stage, (Fig. 4).
Measures 1 mm. long.

The body is very robust. The metasome is rounded anteriorly and slightly produced posteriorly. The anterior margin of the last thoracic segment is convex. The Cephalasome is nearly as long as broad. The segments of the metasome, have the following proportionate lengths.

1	2	3	4	5
45	10	10	5	5 = 75.

The length and breadth of the metasome are 75:45.

The urosome is composed of two segments and is less than a third in length of the metasome. The segments are in the following proportion:

1	2	caudal ramus	
5	9	6	= 20.

The antennule is composed of 17 joints and reaches nearly the end of metasome segment. The joints are in the following proportion:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
45	11	7	6	20	10	18	20	10	12	15	18	25	22	24	16	17

The mouth parts as in the adult but show the usual juvenile characters. The first leg has a two jointed exopod and a single jointed endopod. The other legs have two jointed rami.

First leg: The basal$_2$ carries some teeth on the outer side a long 'S' shaped plumose spine is seen on the inner side as in the adult. The first joint of the exopod is nearly as long as the basal joint and has an outer spine. The second segment is nearly twice the length of the first one. It carries an outer spine, one apical spine two apical and three inner setae. The endopod is only half as long as the exopod. Its outer margin is fringed with very fine spinules. It carries one inner and two apical setae. (Fig. 5). In the second leg the exopod as well as the endopod are two jointed. The first joint of the exopod has an outer spine and an inner seta, while the second joint which is nearly twice as long as the first joint has three outer spines, a long apical spine and five inner setae. The endopod is only half as long as the exopod. The first joint is devoid of setae and the second joint is twice as long as the first and has its outer distal corner produced acutely. It carries one inner, two apical and two inner setae. A row of three spines are found on the segment. (Fig. 6). The rami of the third legs are also two jointed. The first joint of the exopod has an outer spine and an inner seta while the terminal segment which is long and broad carries a long apical spine, three outer spines and four inner setae. The endopod is short and its distal outer corner is produced acutely. It carries one apical and two inner setae (Fig. 7). In the fourth leg the second joint of the exopod is long and narrow and has an apical spine which is denticulate and three outer spines The endopod has one outer and five inner setae (Fig. 8).

The fifth copepodite stage collected at Madras agrees with the description given by Chiba *et al.* (*loc. cit*).

Genus *Scolecithricella* Sars.

Scolecithricella ctenopus (Giesbrecht).
Sewell, 1929, pp. 212-215, Text-fig. 79.
Scolecithrix ctenopus Farran, 1936 p. 95; fig. 9.

Occurrence: Two females from the plankton collected on the 18th December 1953.

Distribution: The great Barrier Reef of Australia (Farran), Malay Archipelago (Scott), Indian Ocean (Sewell), Madras (Present record), the Mediterranean Sea (Giesbrecht) and the Gulf of Guinea (Scott).

Measures 1.23mm long. Body moderately robust with the posterior ends slightly tapering. The end of the last thoracic segment is produced acutely (Fig. 9). The furcal ramus is short and carries four apical setae (Fig. 10). The genital segment is slightly swollen and the genital orifice as shown in the figure (Fig. 11). The antennule is composed of twenty three segments and closely agrees with the description given by Sewell (loc. cit). In the fourth leg, the basal segment is very long and has four small spinules on its inner side and a row of denticles near the distal end. $Basal_2$ is short and has two inner spines. The exopodite is three jointed. The first joint is very short and has a short outer spine. The second joint which is long bears an outer spine and an inner seta. Small denticles are seen towards the inner side. The terminal joint is nearly three times as long as the second one and has three outer spines, a long apical spine, one apical and three inner seta. The two-jointed endopod is short, reaching upto the second exopod joint. The basal joint is very short. The distal joint has a number of spinules and an inner and three apical setae (Fig. 12). The fifth leg (Fig. 13) agrees with the figure given by Sewell (loc. cit.).

Family Pontellidae.
Genus *Pontellina* Dana.

Pontellina plumata (Dana).

Pontellina Plumata, Giesbrecht, 1892, p. 497, pl. iv, fig. 11, pl. xxv, figs. 4, 8, 26, 36, pl. xl, figs. 49-53.

Sewell, 1947, p. 251.

Occurrence: Common in the plankton collected in the months of November and December 1953, especially in the oblique hauls made off Madras.

Distribution: Widely distributed in the Atlantic, Pacific and the Indian Oceans.

HARPACTICOIDA

Family: Miracidae.

Genus *Miracia* Dana.

Miracia minor T. Scott.

T. Scott, 1893, p. 102, pl. xi, figs. 18-30.

Lang, 1947, p. 770, pl. 314, fig. 2.

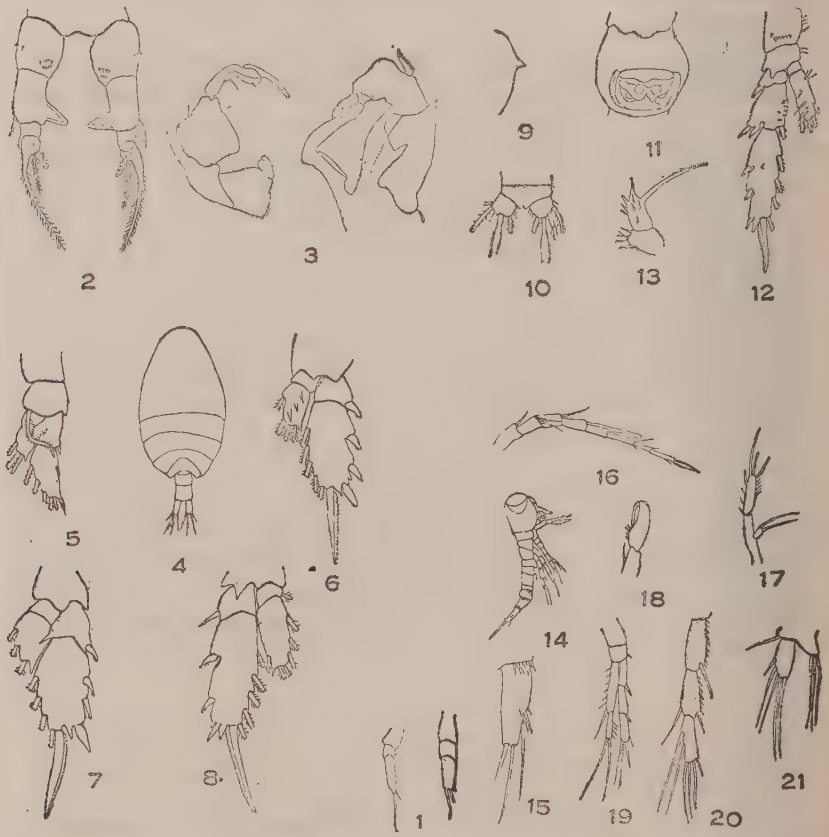

FIG. 1. *Calocalanus plumoloses* male fifth leg (× 400). FIGS. 2-3. *Schmackeria tollingarae*. Fifth leg female, (2), male (3), (× 280). FIGS. 4-8. *Scolecithrix danae* (4), Dorsal view (× 80), 1 to 4 (× 280). FIGS. 9-13. *Scolecithricella ctenopus*. Lateral view of the last thoracic segment, (× 280), (10) caudal ramus, (11) genital segment, (12) fourth leg, (13) fifth leg (× 400.) FIG. 14-21. *Miracia minor*. Lateral view (14), caudal ramus (15), Antennule (16), Antenna (17), Maxillipede (18), first leg, (19) Exopod of second leg (20), fifth leg (21). All the appendages drawn to a magnification of × 400.

The occurrence of this species at Madras is of interest since it is being recorded for the first time from the Bay of Bengal. All the previous records are from the Atlantic and Mediterranean. However, Thompson and Scott (1903) record its occurrence near the gulf of Aden, and Wolfenden (1905) records its occurrence in Maldives. These are the records of its presence in the Indian seas. Steur (1935) has dealt with the distribution of this species and hence its occurrence at Madras is of interest.

Two females were collected from the plankton on the 24th June 1956. A brief description of the female is given below.

Size: 0.9 mm. long.

Body, long and slender, slightly yellowish, with a very prominent cuticular lens which is coloured red. The cephalasome is nearly three times the length of the succeeding segments (Fig. 14). The last three segments of the urosome are fringed with spinules. The furcal ramus is long and slender, three times longer than broad, broader anteriorly tapering posteriorly. It carries two lateral spines and a very long slender seta on its outer margin. There are two apical setae, the inner of the two being nearly twice as long as the outer one (Fig. 15). The antennule (Fig. 16) is eight-jointed, short, reaching only a little beyond the cephalasome. The antenna has a single jointed exopod which is very short and carries two setae apically. The endopod is long and slender, the terminal joint of which bears three inner lateral spines, three spines and a seta apically (Fig. 17). The outer margin is fringed with very fine setae. In the first leg, the rami are long and slender. Basal$_2$ carries a short outer seta. The exopod is three jointed, the first and the second joints carrying an outer spine each while the terminal joint has one outer spine and three apical setae. The first joint of the endopod is very long and slender, reaching upto the second joint of the exopod. It carries an inner seta at its distal end. The terminal joint which is only a third in length of the previous joint carries two setae apically (Fig. 19). The exopod of the second leg is slightly longer than the endopod. The first exopod joint carries an outer spine, the second joint an outer spine and an inner seta and the terminal joint an outer spine, a plumose apical spine and two inner and two outer setae. The second article of the endopod is armed with two inner setae while the terminal one has an outer, an inner and three setae apically (Fig. 20). The third and the fourth legs resemble the second one. The outer margins of all the legs are fringed with fine spinules. The basal

expansion of the fifth leg bears an outer and three inner setae. The third and the fourth legs resemble the second one. The distal joint is nearly twice as long as broad and has four outer and two apical setae (Fig. 21).

CYCLOPOIDA

Family Oithonidae.
Genus *Oithona* Baird.

Oithona spinulosa Lindberg.

Lindberg, 1947, pp. 129-130, Text fig. 7.

Occurrence: Several examples of both the sexes from the plankton from July to January.

Distribution: At present known from Madras only.

Family Oncaeidae.
Genus *Oncaea* Philippi.

Oncaea venusta forma *venella* Farran.
Farran, 1929, p. 284, fig. 33.
Sewell, 1947, pp. 263-264.

Distribution: Off New Zealand and the Great Barrier Reef (Farran), Atlantic Ocean (Farran), Arabian Sea (Sewell), the English Channel (Norman and Scott), and Madras (Present record).

Family: Sapphirinidae.
Genus: *Sapphirina*. Sp. Thompson.

Sapphirina scarlata Giesbrecht.
Giesbrecht, 1892, p. 620, pls. 52-54.
Lehnhofer, 1929, p. 306, figs. 38-40, 42.
Wilson, 1932 p. fig.
Dakin and Colefax, 1940, p. 109, fig. 173 a-c.

Occurrence: Two females from the plankton collected in July, 1956.

Distribution: The Great Barrier Reefs of Australia (Farran), the coast of New South Wales (Dakin & Colefax), Malay Archipelago (A. Scott), Bay of Bengal (Present record), Californian Coast (Esterley), Galapagos Is. (Giesbrecht), S. Atlantic (Cleve).

This is the first record of its occurrence at Madras.

Sapphirina angusta Dana.
Giesbrecht, 1892, p. 620, pl. lii, figs. 5, 6; pl. liii; pl. liv.
Lehnhofer, 1929, pp. 275, 315, figs. 1, 2, 47; 48.
Sewell, 1947, pp. 265-266.

Occurrence: Five specimens (all females) from the oblique hauls made in December 1952.

Distribution: This copepod is widely distributed in the Pacific, Atlantic and Indian Oceans. This is the first record of its occurrence at Madras.

Sapphirina nigromaculata Claus.
Lehnhofer, 1929, pp. 304, 336, figs. 38-41; 65; 66.
Sewell, 1947, p. 267.

Occurrence: A single male from the plankton collected on 6-2-1956.

Widely distributed in the Pacific, Indian and Atlantic Oceans.

Sapphirina sinucauda Brady.

Sapphirina auronitens sinucauda Lehnhofer, 1929, pp. 289, 329, figs. 22-28.

Sewell, 1947, pp. 268, 269.

Occurrence: A single female from the plankton collected in July, 1955.

Distribution: It is widely distributed in the Pacific and Atlantic Oceans. In the Indian Ocean it has been recorded from the Ceylon Pearl Banks (Thompson and A. Scott), the Bay of Bengal (Present record) the Laccadive Sea, the Arabian Sea (Sewell), the Red Sea (Steur), and East Coast of Natal (Cleve).

Genus *Copilia* Dana.

Copilia mirabilis forma *Platyonyx* Lehnhofer.
Lehnhofer, 1926, p. 127, Figs. 4, 5-7, and 18.
Sewell, 1947, p. 271.

Occurrence: A single male from the plankton collected in July. Widely distributed in the tropical Pacific, Atlantic and Indian Oceans.

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The Food and the Feeding Habits of Some Madras Fishes*

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ABSTRACT

A biological and volumetric analysis of the stomach contents of 631 fishes of three species belonging to three families namely *Mullidae, Sciaenidae* and *Sillaginidae* is given. All the three fishes studied are carnivorous in habit. The adults of *Upeneus cinnabarinus* visit the sea bottom occasionally to feed on mud rich in organic matter while those of *Sillago sihama* feed at the surface and bottom of shallow areas and *Sciaena sina* appears to confine its feeding to mid water levels only. These three fishes breed away from the Madras inshore waters and use the Madras inshore waters as feeding grounds.

Introduction

Though a study of the food and feeding habits of fishes will bring to light a considerable data regarding seasonal scarcity, shoaling and migration and help commercial fisheries in forecasting the movements of fish into or out of their feeding grounds yet at present we know very little regarding the food and feeding habits of fishes of market value. Practically nothing is known regarding the diet of *Mullidae, Sciaenidae* and *Sillaginidae* which form a good proportion of the fishes sold in Madras market. Therefore a study of the stomach contents of three fishes (*Upeneus cinnabarinus, Sciaena sina* and *Sillago sihama*) belonging to these families was made. A detailed account of the history of the previous workers has been already dealt with by the author (Kuthalingam, 1955 a).

* Formed part of the thesis approved for the degree of Master of Science of the University of Madras.

Material and method

A total number of six hundred and thirty-one fishes have been examined in the laboratory during the period of investigation. They were chosen because they are cheap and common in the market and are available almost throughout the year. A detailed description regarding the method of determining the volume of food has been already described by the author (*loc. cit.*).

Upeneus cinnabarinus: This Red mullet belongs to the family Mullidae of the order Acanthopterygii and has the general features characteristic of the family. The most outstanding feature of this Goat-Fish is a pair of stiff barbels below the chin. When swimming along the bottom the barbels are carried in advance to feel the way. It is commonly known as "Navarai" in Tamil. Colour:—"Vermilion, darkest on the back; there appears to be a central silvery spot in the middle of each scale in two rows above and the two below the lateral line. Dorsal and anal rays yellow, upper caudal lobe orange, the lower one red. A large purple spot covers the opercle and descends on the subopercle" (Day, 1889). Distributed in the seas of India and Ceylon where it is said to be abundant. In Madras coast it is netted from July to January of each year. It is probable that this fish migrates away from Madras waters after January in search of fresh feeding grounds.

189 adult specimens were examined from July 1953 to January 1955 of which 14 were without food in the stomach. These 175 had food in the stomach; out of which 33 were full fed, 40 were ¾ fed, 32 were ½ fed, 32 were ¼ fed and 38 were ill-fed. The fishes examined ranged between 11 cm. to 16.5 cm. in length, only 14 being 11 cm. in length and 22, 16.5 cm. in length. There were 125 females of which 80 were mature and 64 males of which 23 were mature. The analysis of the stomach contents showed no difference between male and female, mature and immature fishes, as regards diet. This fact is noteworthy since diet differs in the mature and immature stages of certain other species like *Upeneus indicus, Caranx djedaba,* etc.

The undigested and identifiable matter of the stomach contents was measured and analysed into different constituents. The volumes of the different constituents calculated in percentages of the total volume of identifiable food are presented below as monthly averages.

TABLE I

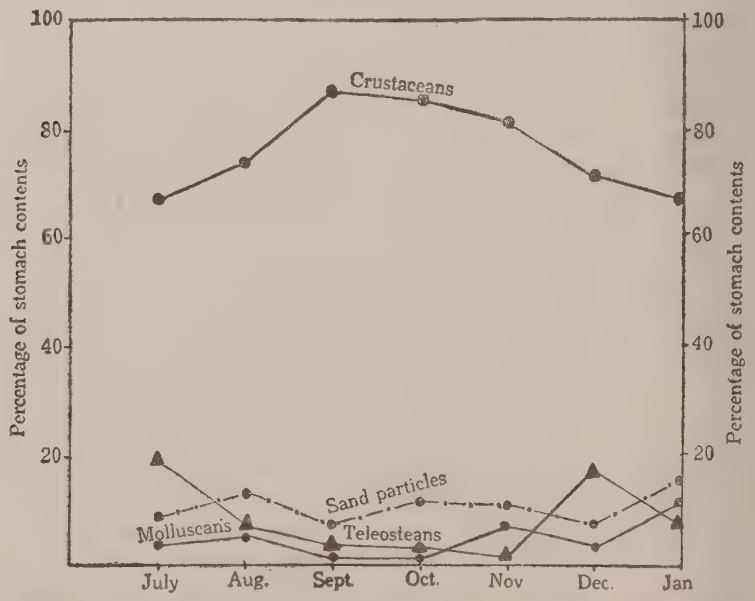
Table showing the monthly average of total volume of food and the percentages of the different constituents of *Upeneus cinnabarinus*.

Particulars	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.
Vol. of stomach contents in c.c. ..	0·3	0·7	0·5	0·6	0·6	0·6	0·7
Crustaceans ..	66·9	74·0	87·1	85·2	81·4	71·7	66·3
Teleosteans ..	19·9	6·1	3·4	2·9	1·7	17·5	6·1
Molluscans ..	3·8	6·4	1·0	0·7	6·2	3·5	10·5
Sand particles ..	8·6	12·9	7·2	11·2	10·7	7·3	14·0
Miscellaneous food items ..	0·8	0·6	1·3	—	—	—	3·1

These fishes are strictly marine and carnivorous in their feeding habits. From the table No. III it is quite evident that crustacea form the main bulk of the diet. Large fast swimming forms like *Squilla mantis, Acetes erythraeus, Metuta victor,* prawn and cumacea form the main bulk and small forms like megalopa, zoea stages of crab form only a small portion. The teleostean food item consisted of not only planktonic eggs but also of larva and juveniles belonging to *Clupeidae* and *Engraulidae* which appear to be the most frequent teleostean food on which the fish feeds. About 4% of the total volume of food was of mollusca which consisted of larval bivalves and gastropods and also broken shell pieces. There were no polychaetes or their remains in any fish throughout the period of investigation. This was all the more remarkable because the polychaetes formed more or less a regular food item of the related species *Upeneus indicus* during the corresponding periods. Further the present species *Upeneus cinnabarinus* is a fish which frequents the benthic region of the sea and sand particles formed nearly 10·5% of the stomach contents. The presence of sand particles is not likely to be secondary. It is also possible that these fishes feed on sand and subsist on the organic matter mixed up along with the sand. Hence the omission of polychaetes from the menu is highly suggestive of its feeding selectively. This is further confirmed by the fact that the crustacea

which form 76·6% of the food are all larger forms which have to be chased and captured. Owing to the absence of planktonic organisms in the stomach contents of these fishes and owing to the presence of nektonic organisms as well as the benthic forms it may be inferred that it feeds between midwater and the bottom, never rising to the surface waters.

The data regarding the volume of stomach contents throw light on the rate of feeding during the six months this fish sojourns in Madras inshore waters. On the whole the monthly averages are more or less uniform suggesting a steady rate of feeding. Though we know that the food available in inshore waters is far more abundant in November and December than in other months, this fish feeds more or less steadily throughout all the months. This is to be expected only in the case of selective feeder of predatory capacity. Even when large crustacea are fewer it can secure its volume by chasing and capturing.



GRAPH No. I. Monthly variations in the proportions of the food components of *Upeneus cinnabarinus* (Cuv. and Val.).

From the graph (No. 1) setting forth the analysis of the data of the stomach contents it will be evident that crustacea which form the main bulk of this fish exhibit a gradual increase from

July to October and remain more or less uniform till November whereupon a gradual decline in the crustacean food might be followed to January. It may also be seen from the graph that while the crustacean food item increased the molluscan item as well as that of the sand particles showed a decrease during the months of July to October. The slight decline in the crustacean item in the month of November appears to have been compensated by a corresponding increase of the molluscan and sand items, while in December the teleostean item increased. By January the crustacea and teleostei reached a minimum and the fall was compensated by the molluscan item and sand particles. It is possible that the fall in crustacean item between October and January is due to the onset of breeding season when the larvae of crustacea become planktonic rising to the surface and that *Upeneus cinnabarinus* being a fish never rising to the surface on the other hand prefers to reach the bottom and feed on mollusca and sand particles. The fish leaves the Madras area because the larger crustacea which it prefers become fewer in December and January.

Sciaena sina: This fish belongs to the family Sciaenidae of the order Acanthopterygii. Commonly known as "Kathali" in tamil. Colour: "Silvery tinged with the brownish along the back and shot with gold on the abdomen, first dorsal blackish especially in its outer half, the other fins grey" (Day 1889). Distributed in seas of India. Bapat and Bal (1952) studied the food of young Sciaenids like *Sciaena miles, Sciaena albida, Sciaena semilactosa* and *Sciaena glauca* and found that the younger forms of all the *Sciaenids* take prawns as their main food, and that the percentage of their fish food goes on slowly increasing as they grow in size. The present study is based on an analysis of stomach contents of adult fishes only.

An examination of the stomach contents of 264 fishes was made and the data collected from 1953 July to 1955 May. Out of this, 234 fishes had food in their stomach and the rest were found empty. 60 fishes had full stomach, 38 fishes were ¾ full, 40 fishes were ½ full, 48 were ¼ full and 48 fishes had little food in the stomach. The fishes ranging between 9 cm. to 25 cm. in length show no difference in their food. Although the different size groups of this fish take the same type of food it was found that while the small fishes fed on the smaller crustacea the larger fishes confined themselves to only larger crustacea. There were 180 females of which 68 were mature and out of 84 males 20 were mature and the rest were immature. There was no difference between the mature

and immature fishes as regards feeding. It is quite evident from the data collected that maturity does not in any way affect the feeding habits of these fishes as it does in certain other fishes.

The volumes of stomach contents were measured and analysed into different constituents and the data obtained are tabulated as follows in percentages of the total volume of identifiable food.

Taking only the undigested and identifiable matter of the stomach contents into consideration the monthly average of the various food items are tabulated below.

TABLE II

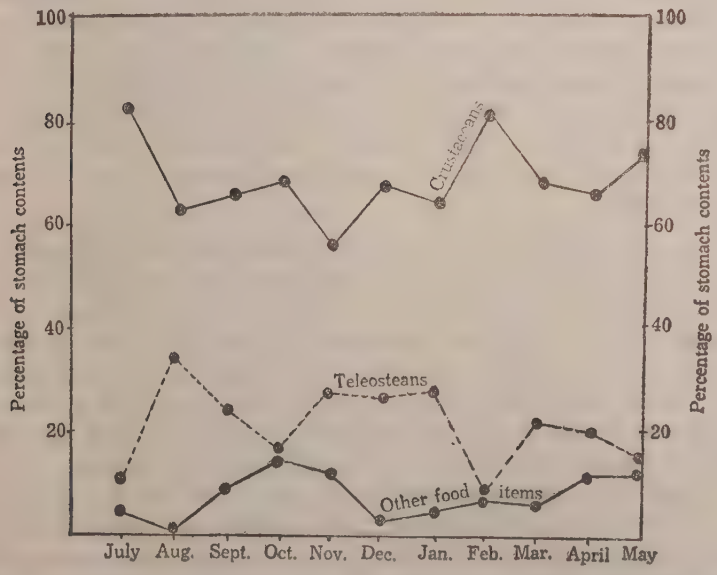
Table showing the average total volume of food along with the percentage average of the various food items of *Sciaena sina.*

Particulars	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
Volume of stomach contents in c.c.	0·7	0·9	0·8	0·7	0·6	1·1	0·8	0·9	0·9	0·7	0·6
Crustaceans	82·8	63·9	65·1	68·1	56·6	68·2	65·8	82·6	69·9	66·3	75·1
Teleosteans	11·7	35·6	25·6	17·4	29·7	27·9	29·1	9·2	23·9	20·5	13·3
Other items	5·5	0·8	9·3	14·5	13·7	3·9	5·1	8·2	6·2	13·2	11·6

It is evident from the table (No. II) that crustacea form the main food item of this fish. Of the crustacea *Squilla holoschista, Acetes setiferous, Acetes erythraeus, Penaeus indicus, Penaeus carinates, Metapenaeus monocerous, Emerita,* Amphipods, Alima larva, *Charybidis annulata* form the main constituents.

There were also larval crustaceans such as zoea, and megalopa stages. Apart from this crustacean item there were also other unidentifiable appendages and other remains of crustacean. Teleostei form 22·6% of the total volume of food. Species of *Engraulis, Clupea, Upeneus, Sciaena* and *Dussumieria* were the most frequent fish-food the fish prefers. These forms were identified as *Clupea toli, Upeneus indicus, Umbrina dussumieria* and *Dussumieria acuta.* There were also post larval eels and other

partly digested fishes which could not be identified. Apart from this, fish-eggs, scales, pectoral girdle, fins, bits of vertebral column and other remains of teleosts were found. One of the fishes 13 cm. long caught on 13th October, 1953 had among other gut contents the remains of another large fish which could not be identified. The most notable of the remains was the entire alimentary canal which measured 9 c.c. and contained a juvenile fish of the family *Leiognathidae*. Since the juvenile fish was almost undamaged though the rest of the predator had been considerably affected by the digestive juices it may be inferred that *Sciaena sina* should have swallowed the fish whole in live condition and not when it was dead and decaying at the bottom. Remnant pieces like parapodia, isolated segments and setae of polychaetes formed the rest of the gut contents. Since these polychaetes are pelagic in habit and since sand particles formed only a very negligible quantity it may be inferred that this fish is not a bottom feeder. It is actively predaceous at midwater levels of the sea. The sand found in the stomach must have come from the guts of crustacea or from other fish prey. There were also very small quantities of green digested matter, bivalve shells and other unidentifiable remains.



GRAPH No. II. Monthly variations in the proportions of the food components of *Sciaena sina* (Cuv. and Val.).

From the graph (No. 2) it will be seen that the fish takes a maximum quantity of crustacea in the months of July and February, and in the month of November the crustacean food showed a decrease, which is compensated by the teleostean food item. It is also evident from the graph that the fish prefers crustacean diet rather than teleostean food. The sudden fall in the crustacean food item in the month of August is compensated by the teleostean food. The teleostean food reached its peak in the month of August and showed a sudden fall in the month of February, whereas the decrease of teleostean food item in the month of October is compensated by the increase of other food items such as the vegetable matter, mollusca and polychaetes. Thus there is a correlation kept up by the fish throughout the year as is seen from graph (No. II).

Regarding the volume of stomach contents during these months we find that this fish feeds most in the month of December and least in the month of May. It is probable that the scarcity of food preferred by the fish, is responsible for the fish leaving the Madras waters for other feeding grounds.

Sillago sihama: This fish belongs to the family Trachinidae of the order Acanthopteryii. Known as "Kelankan" in Tamil. Colour:—"Olive green along the back, becoming light on the abdomen, the whole having a brilliant purple reflection, a silvery longitudinal band running along the body" Day (1889). Maximum length attained is a foot. Distributed in Indian seas to Malay Archipelago. This whiting is both marine and estuarine and comes into very shallow waters—(Devanesan and Chidambaram, 1953).

A total number of 178 fishes were examined in the laboratory from September 1953 to May 1955. After May, not even a single fish was landed in Madras coast. Except 18, all had food in the stomach. Out of 160 fishes examined, 20 were fully fed, 30 were ¾ fed, 42 were half-fed, 32 were quarter-fed and 36 were poorly fed. Fishes examined ranged between 9·5 cm. to 18·5 cm. in length out of which 18 were 9·5 cm. and 23 were 18·5 cm. and the rest were in between the two. There were 96 females, out of which 48 were mature and out of 64 males 33 were mature, and the rest were found immature. The mature fish seem to have fuller stomach than the immature ones, although there is a possibility that these immature fishes might have been examined long time after their feeding was over. But such a possibility is to be

ruled out since the diminished food contents in the stomach had been a regular feature on all occasions whenever they were examined. The following table will illustrate this.

TABLE III

Table showing the condition of food and also the length of the fishes examined during mature and immature stages of *Sillago sihama*.

Condition of food ↓ / length of fishes examined. ↑	9·5 cm. to 11·5 cm.		12·5 cm. to 14 cm.		15·5 cm. to 18·5 cm.	
	mature	immature	mature	immature	mature	immature
Empty ..	1	4	3	7	—	3
Little ..	4	12	3	10	2	5
¼ ..	3	8	8	6	4	2
½ ..	12	3	10	4	12	1
¾ ..	8	2	7	3	8	2
Full ..	4	—	5	—	9	2

The stomach contents were measured and analysed into different constituents and the data obtained are tabulated as follows:

TABLE IV

Table showing the average volume of food and also the percentage average of the various food items of *Sillago sihama*.

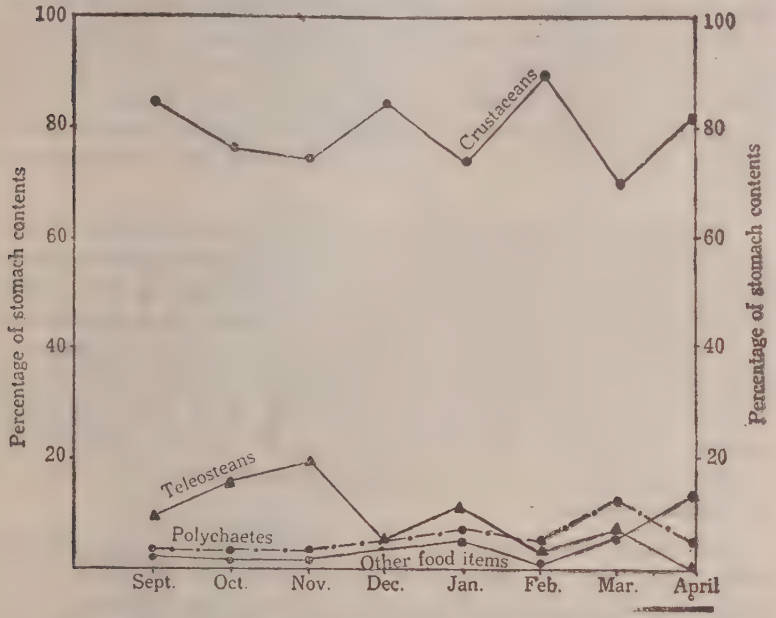
Particulars	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Vol. of stomach contents in c.c. ..	0·5	0·5	0·5	0·4	0·4	0·6	0·4	0·5
Crustaceans ..	84·3	76·8	75·5	85·1	74·3	90·4	71·6	82·8
Teleosteans ..	9·1	15·8	19·0	5·1	7·0	4·3	12·0	4·1
Polychaetes ..	3·8	3·8	2·9	5·1	10·4	2·7	7·5	—
Sand particles ..	1·0	1·2	1·3	1·2	3·5	1·4	2·6	—
Other items of food ..	1·8	2·4	1·3	3·5	4·8	1·2	6·3	13·1

It is evident from the table (No. IV) that crustacea form the main food item of this fish. Of the crustacea *Penaeus sp, Squilla sp, Acetes sp, Lucifer, Emerita sp,* and copepods form the main bulk of the food. Copepods were identified as *Eucalanus crassus, Acrocalanus longicomis, Paracalanus parvus,* and *Acartia erythracea.* Apart from this there were also other unidentifiable appendages and other crustacean remains. Species of larval and post-larval forms of *Clupea* and *Engraulidae* were found in the stomach. Fish scales, eggs, vertebral column and small bones were also found along with the stomach contents. There were evidences of the fish feeding on polychaetes. The stomach of one fish caught on 10th January, 1954 was exclusively filled with *Nereis.* On certain occasions numerous tubes of the tube-dwelling polychaetes were found inside the stomach. Polychaetes belonging to the families *Maldanidae* and *Glyceridae* were identified. Since the polychaetes belonging to these families are bottom-dwelling forms it must be concluded that *Sillago sihama* frequents the bottom layers of the sea. In majority of cases specific identification was not possible, the only recognisable parts were setae. Since the fish feeds on polychaetes, it is probable that the fish might have taken sand particles also along with the polychaetes and this is supported by the fact that in the month of April the fish has not consumed polychaetes at all, and hence no sand particles were found in the stomach in that month. From this fact it is concluded that though sand particles were present in small quantities, their presence was only secondary. The stomach contains small quantities of green matter mixed with mucus. Algae and diatoms were the only vegetable stuff in the diet of the fish. The quantity of algae consumed was greater than the diatoms. It was not possible to identify the algal filaments as they were always mixed up with mucus and partially digested. The diatoms identified were *Chaetoceras, Thallasiothrix* and *Coscinodiscus.*

In view of what Devanesan and Chidambaram (1953) had observed that Balanoglossus was found in the stomach of this fish which they presumed as having been dug out of the sand with the aid of the snout, it is probable that this fish adopts the same mode of feeding with reference to polychaetes since sand also appears to be swallowed along with food.

From the graph (No. III) it is seen that the crustacean food reached its maximum in the month of February and a fall is observed in the month of March. The fall in crustacean items

in the month of March is compensated by the increase of Teleosts, Polychaetes and other miscellaneous food items. Similarly in



GRAPH No. III. Monthly variations in the proportions of the food components of *Sillago sihama* (Forsk).

the month of January the fall in the crustacean item is compensated by Teleostea, Polychaetes and other food items. So it is probable that whenever the crustacean food becomes scarce the fish as a substitute prefer the other food items.

Discussion

From the data presented it will be obvious that none of the three fishes studied is a typical surface feeder; *Upeneus cinnabarinus* appears to feed on animals at the sea bottom occasionally but most in mid-water; *Sillago sihama* whose diet consists of surface as well as bottom food appear capable of swimming up and down the shallow inshore waters of Madras and *Sciaena sina* appear to confine its movements to midwater levels only.

It has been observed by Russel (1929) and Raymont (1923) who studied the feeding habits of fishes belonging to the families *Clupeidae, Gadidae, Bothidae,* and *Pleuronectidae* from temperate

regions that these fishes choose their diet and thereby indicate a capacity for taste as well as an ability to secure the food which these fishes prefer. Vijayaraghavan (1950) found evidences of such a preference in choosing their food even in plankton feeding fishes like *Pellona, Sardinella* and *Dussumieria.* In the present study it was found that though *Upeneus cinnabarinus* feeds on other organisms at the sea bottom it selectively omits Polychaetes which form a regular item of diet of *Upeneus indicus* (Kuthalingam, 1955a).

Excepting the following fishes Viz. *Caranx djedaba, Sillago sihama* and *Upeneus indicus* (Kuthalingam, 1955 thesis) all the fishes examined showed no difference in their feeding with regard to maturity stages. In other words maturation does not in any way affect the feeding habits of these fishes. It is of interest to note that *Caranx djedaba* (Kuthalingam, 1955b) and *Sillago sihama* feed less during immature stages than when they are mature (vide table No. III) where as in *Upeneus indicus* the volume of stomach contents of immature fishes is greater than in its mature phases.

All these fishes examined are carnivorous in their feeding habits and feed on all prey such as crustacea, teleostei, larval bivalves and gastropods which happen to occur in large quantities. Whenever molluscan shells and sand particles were found in the stomach, large amounts of profuse mucus secretion formed a slimy coating on those materials as has been observed by Job (1940) and Vijayaraghavan (1950). Job (loc. cit) suggests "one of the main purposes served by the viscid mucus is to form slippery protective investment for the delicate gastric mucosa and to bind the food contents into a smooth yielding mass".

The damage caused to fisheries by the piscivorous feeding habits of some of the fresh water Perches had been recognised as early as in 1880 by Gunther. Vijayaraghavan (1950) observed that *Trichiurus haumela* and *Trichiurus savala* which feed on clupeoids, thus causing a damage to the clupeoid fisheries; where as the present investigation shows that *Upeneus cinnabarinus* feed on the (post larval and juvenile forms belonging to the) families *Clupeidae Engraulidae, Polynemidae* and *Leiognathidae* of market value, it may be concluded that this Red mullet is harmful to fisheries.

Sillago sihama, Sciaena sina and *Upeneus cinnabarinus* use the Madras inshore area for feeding purposes only. The adults of

all these species leave Madras waters, when the food becomes scarce, for feeding and spawning purposes elsewhere. The eggs and larvae of none of these forms have met with in townet plankton. *Upeneus cinnabarinus* arrives in July and leaves in January where as *Sillago sihama* visits the inshore waters in September and leaves in March and *Sciaena sina* leaves in May and comes back only in August.

Summary

1. The food and feeding habits of the adults of *Upeneus cinnabarinus, Sillago sihama* and *Sciaena sina* were studied and the results presented. Since the juveniles of these species were not obtained only the diet of the adult has been discussed.

2. The results of the study of the above mentioned three species belonging to three different families are discussed with reference to :

a. The level at which these fishes feed in the sea;

b. Selective feedings;

c. Feeding in relation to maturation;

d. The different animals which form the menu of these carnivors;

e. The harmful effect on inshore Fisheries.

Acknowledgement

I wish to thank Dr. C. P. Gnanamuthu, Director, University Zoology Research Laboratory, Madras for suggesting the subject and constant encouragement and guidance during the course of the work which was done in the zoology laboratory of the Madras University.

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Regeneration and Growth of Mutilated Pieces of the Colony of *Polyclinum Indicum* Sebastian

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ABSTRACT

Colonies of *Polyclinum indicum* Sebastian were sliced in thickness of 2mm., 5mm., 1cm. and above, and were kept on glass slides in sea water inside laboratory troughs. All the 2mm. pieces died, while most of the 5mm. pieces survived. It appears that regeneration was possible only if a sufficient number of uninjured zooids were retained inside the pieces. The same type of pieces when enclosed in wire gauze cages and immersed in the sea showed that many pieces of 2mm. size survived which was probably due to the higher percentage of oxygen present in the natural habitat. Regeneration of individual zooids was by budding depending on the level of the injury sustained; if the cut was through the thorax and abdomen budding was possible, whereas if the cut was through the post-abdomen they underwent autolysis. When portions of a colony were cut and kept separate, both the parts regenerated by budding, and finally retained the shape of the colony.

Introduction

That Ascidians have the power to heal an injury or regenerate lost parts is well-known. If the portion of a zooid is cut away, the lost parts develop from the remaining portion. In extreme cases, the whole zooid develops from a fragment of the stolon as is found in asexual budding. Experiments on the regeneration of lost parts of ascidian zooids have been conducted in *Ciona* by Schultz (1899), Hirschler (1914) and Pérès (1948b); on *Polycarpa* by Selys-Longchamps (1915); on *Styela* by George (1937); on *Perophora* by Deviney (1934); on *Clavelina* by a series of authors: (Brien, 1930 a, b; Berrill & Cohen, 1936; Fischer, 1937; Harrisson & Pasquini, 1930; Pasquini, 1933, '34, '37; Pérès, 1948a). In all cases the branchial lining, peribranchial lining, epidermis or epicardium initiates the regeneration of lost parts according to the rate of injury. A similar phenomenon is also found in the case

of dedifferentiation of zooids under adverse conditions and further reconstitution. The latter depends upon the amount of regression; if only partial, the zooids are reconstituted from the remaining parts, if severe, from the epicardium, and in extreme cases, re-differentiation is initiated by a small package of cells. These phenomena are well exemplified by the studies on *Clavelina* by Driesch (1902), Ries (1937 a, b), Huxley (1926) and Spek (1927), on *Perophora* and *Amaroucium* by Huxley (1921), and on *Botryllus* and *Botrylloides* by Berrill (1941 a, b & '47). The dedifferentiation and reconstitution in a tropical ascidian *Polyclinum indicum* has been studied by Sebastian (1954 a, b). While the aim of the organs or groups of cells in mutilated, independent zooids is to reconstitute the lost parts or regressed zooids to its original shape, in a colony where individuals zooids are embedded inside a common test, and hence have no independent existence, the process of regeneration will depend on the relation of the individuals zooids to the colony as a whole. The present paper deals with the process of regeneration of zooids inside the test of the colony of *P. indicum,* and the regaininng of the original shape of the colony as a whole.

Experiments

Four different experiments were conducted to study, (1) the relation of the size of the fragments of a colony to its capacity for regeneration, (2) the capacity for survival of sliced colonies in natural environment, (3) the mode of regeneration of mutilated zooids inside the test, and (4) the mode of regaining the shape of the colony after mutilation.

Experiment 1. *Relation of the size of the fragments of the colony to its capacity for regeneration*: (Following Herdman (1888) the terms length, breadth and thickness, are used in this section to dimensions indicated in Fig. 1). The colonies were cut lengthwise as shown in Fig. 2, the breadth of slices varying from 2 mm., 5 mm., 1 cm. and above 1 cm. The colonies chosen for slicing were such that the length and thickness were more or less of the same dimensions. When sliced, the edge pieces like AA′ will have only one side cut, while the other pieces, BB′, CC′, DD′, EE′, FF′ will be cut on both sides, and the zooids inside the test will be cut along different levels according to their dispositions. The area of the cut surfaces of slices A, B, C, D, E, F will be equal to those of A′, B′, C′, D′, E′, F′, though the perimeter of the different slices A to F will vary according to their position in the colony, (F will

be as shown in Fig. 3). In a slice of 1" long, 1" thick and 2 mm. broad, the area of the cut surfaces on each side of the slice will be approximately less than 5·07 sq. cms., while the area of the exposed outer side where zooids open will be about 1·59 sq. cms. These areas decrease slightly from the middle to the edge pieces. But the area of the exposed uninjured surfaces of AA′ with only one of the sides cut will be more than in other types of pieces, and consequently lesser number of zooids will be injured.

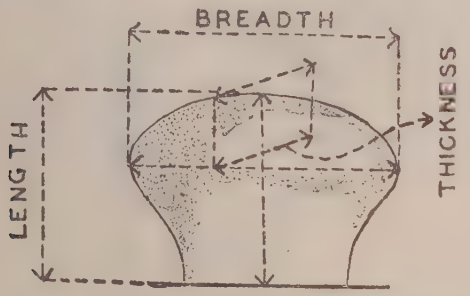


FIG. 1.

FIG. 2.

Several such pieces were left flat on glass slides resting on cut surfaces, and were kept in troughs of sea water. (Fig. 4 shows one of the middle pieces, and Fig. 5, the edge piece kept on glass slides). The slices were examined every 24 hours to see if they were alive or dead, the colour of the test (Sebastian '54 b) becoming yellow when the slices were dead. Out of the 36 pieces of AA′ type only 6 survived, while all the 109 pieces of the remaining types perished. It is obvious that in slices AA′ there is proportionately greater amount of free outer surface of the colony, and therefore a larger amount of zooids opening into it. Since in

these slices the area of the single injured surface is much less than in other slices like BB′-FF′ and lesser number of injured zooids, at least six out of the thirty-six slices tested were able to live after twenty-four hours resisting bacterial action. Such pieces are able to regenerate by undergoing the process of asexual budding.

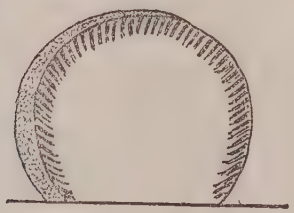


FIG. 3.

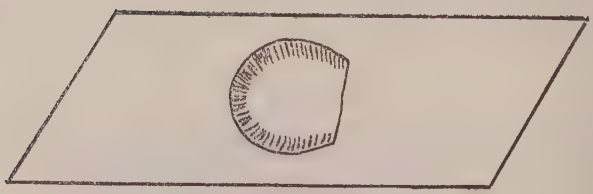


FIG. 4.

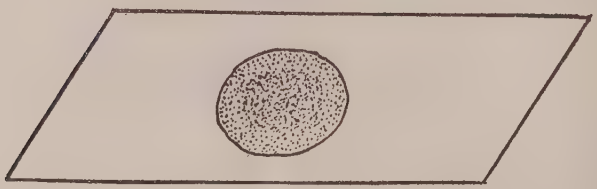


FIG. 5.

Another colony half the size of the first was sliced in the same way and left in sea water. It was found that 12 out of 32 slices of the AA′ type survived while the rest perished. The increase in number which survived (12) when compared to those in the previous experiment (6) may be because, the colony being smaller and younger, the zooids are also smaller in size, and hence lesser proportion of them sustain injuries while cutting than in larger and older colonies possessing zooids of larger dimensions. The

remaining types BB′, CC′, DD′, DD′, EE′, FF′ died in spite of the fact that the area of the cut surface was less (1.28 sq. cms.), which indicates that when two surfaces are injured the proportion of uninjured zooids in smaller colonies also may not be sufficient to initiate further growth.

In order to see if increasing the breadth of the slices would favour regeneration, colonies 1″ and ½″ size were cut into slices 5 mm. broad, and left in the sea water as before. From the results tabulated in Tables I and and II, it will be seen that a larger number of the AA′ type survived while a varying number of the other types also continued to live. From this experiment it is obvious that though the area of the cut surfaces may be the same as before, yet if the breadth of the slice be increased, the number of zooids left intact being greater, the chances of the recovery are increased.

TABLE I.

(Slices 1″ × 1″ × 5 mm.)

Types of slices.	No. of slices observed.	Number of slices survived.
AA′	29	19
BB′	28	9
CC′	31	12
DD′	24	11
EE′	29	9
FF′	27	8

TABLE II.

(Slices ½″ × ½″ × 5 mm.)

Types of slices.	No. of slices observed.	Number of slices survived.
AA′	43	38
BB′	39	29
CC′	42	37
DD′	40	30
EE′	35	26
FF′	35	28

Experiment 2. *Survival of sliced colonies in natural environment*: In order to see if sea water from natural habitat, Royapuram coast of Madras, will be more favourable for the regeneration of slices than sea water from Chepauk area, which is used for laboratory experiments, colonies 1″ and ½″ sizes were sliced 2 mm. broad as in the previous experimennts and left inside wire gauze cages immersed in the sea, in the natural habitat (Royapuram area of Madras coast). Though in this experiment both the cut surfaces of the slices will be exposed to the action of sea water, other conditions were more or less the same. However, as can be seen from Table III, even 2 mm. broad pieces of AA′ type show greater proportion of recovery, while slices of BB′, CC′ and DD′

TABLE III.

(Slices 1″ × 1″ × 2 mm.)

Types of slices.	No. of slices observed.	Number of slices survived.
AA′	28	15
BB′	32	9
CC′	20	5
DD′	22	2
EE′	21	—
FF′	19	—

TABLE IV.

(Slices ½″ × ½″ × 2 mm.)

Types of slices.	No. of slices observed.	Number of slices survived.
AA′	30	22
BB′	31	10
CC′	24	12
DD′	28	10
EE′	25	8
FF′	31	6

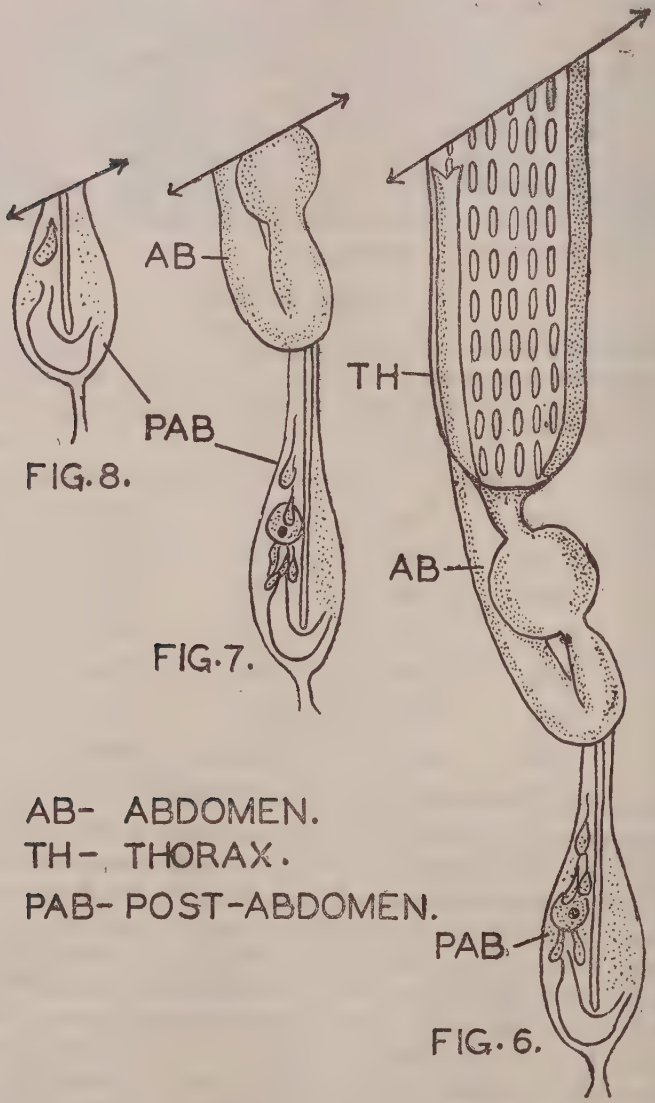
types also show much better recovery than in laboratory conditions. Slices of smaller colonies of ½″ size fare even better (shown in Table IV), some of even EE′ and FF′ types were able to survive. Slices of greater breadth, ½ cm. and 1 cm., showed greater percentage of regeneration than 2 mm. broad pieces. It will be obvious from these that when both the cut surfaces are washed by sea water, the mutilated zooids show higher percentage of recovery and regeneration. Further, as can be seen from Table V, the sea water used for experiments in the laboratory differed from the sea water of the natural habitat in pH and oxygen concentration. It is likely that the higher concentration of oxygen is a factor of importance in the recovery and regeneration of the fragments of colonies.

TABLE V.

Sea water at Chepauk.			Sea water at Royapuram		
pH	Salinity $^0/_{00}$	0_2 c.c. per litre.	pH	Salinity $^0/_{00}$	0_2 c.c. per litre.
8·20	33·00	3·99	8·20	34·01	4·21
8·20	33·98	3·80	8·00	33·20	4·99
8·20	30·48	3·90	8·10	30·20	4·92
8·10	24·49	3·90	8·00	29·40	4·49
8·10	27·39	3·90	8·15	27·10	4·98
8·20	27·80	3·60	8·00	27·00	4·89
8·15	27·40	3·70	8·10	27·00	4·99

Experiment 3. *Regeneration of mutilated zooids within the test of Polyclinum indicum*: In order to determine the significance of the mutilation of the thorax, abdomen and post-abdomen in the regeneration of a zooid, the following experiment was performed. A colony was cut through obliquely as shown in Fig. 9, in such a way that a number of zooids running vertically down from the top of the colony would be sectioned (a) some with part of the pharynx cut off (Fig. 6), (b) lower down some with pharynx and part of the abdomen cut off (Fig. 7), and (c) lower still, some with pharynx, abdomen and part of the post-abdomen also cut off (Fig. 8). If such parts of colonies with mutilated zooids are left

in large tanks of sea water the details of the process of reorganisation can be observed. It is found that the fate of zooids depends on the level of the cut made. The zooids (c) whose post-abdomen are sectioned, do not regenerate but become autolysed and



resorbed. In the other two types (a) and (b) the post-abdomen undergoes changes to form buds. There appears to be a relation

between the number of buds and the level of fragmentation. In a zooid of normal size, if the cut is at a level towards the anterior side of the thorax, generally three buds are formed, while, if it is at the posterior level of the thorax only two are formed usually, but very rarely only one may be formed. The zooids cut along the abdomen produce only one bud as a rule. The buds that are formed develop and form zooid systems, and reappear at the cut surface, and along with their appearance fresh test material is secreted and the colony is set on the road to complete regeneration.

Experiment 4. *The mode of regaining the shape of the colony after mutilation* : In the study of regeneration of zooids of a colony like that of *P. indicum,* the restoration of shape or the contour of the colony is of considerable interest. This is particularly so in *P. indicum* where the zooids are confined to the periphery within the test and the interior occupied by only the ampullae. In order to trace the steps by which the shape of the colony is regained, several colonies were cut along an oblique plane forming an angle with the broad axis of the colony, as shown in Fig. 9. By this, a small piece B is sliced off leaving the major part of the colony A left attached to the substratum. Along the cut edges of parts A and B the zooids have been segmented at various levels. In the segment A, in about ten to fifteen days, the mutilated zooids along the cut edge regress and reorganise into new zooids. These zooids become pushed upwards into the water at right angles to the cut edge so as to form a ridge-like elevations, as shown in Fig. 9 A1. Later, all the zooid systems of the entire colony pass into the asexual phase, each zooid producing three buds, thus the number of zooids newly pushed up along the cut edge also increasing three-fold. In this way after every asexual phase of reproduction the cut surface becomes more reduced than before, till it is completely covered over, (Fig. 9 A2 and A3), and the injured colony regains more or less the original shape.

If the piece B is placed with the cut edge on the substratum it acquires the shape of the colony in the following manner. Around the edge touching the substratum the zooids have been cut at various levels leaving the anterior severed portions inside the test. Such portions autolyse and become resorbed. As a consequence, the test near the edge of the place becomes weakened and being thinner towards the periphery than towards the middle of the piece the edge curves inwards. About this time the cut surface gets fixed to the substratum. The zooids in the outer un-

injured part of the piece have their post-abdomens intact and they help the growth in size of the colony by the production of new buds as can be seen from the Figs. 9 B1 and B2.

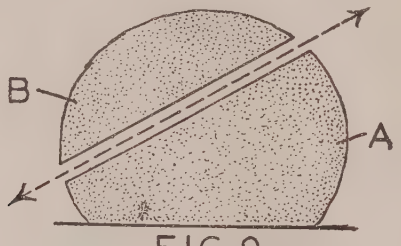
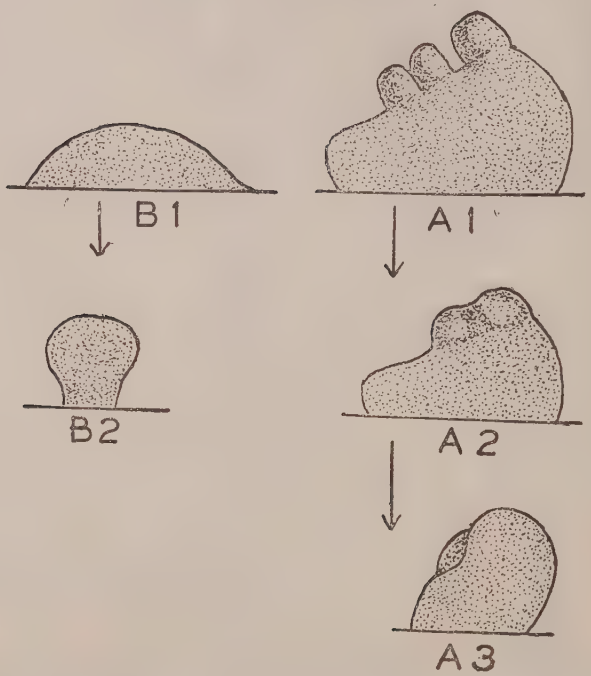


FIG. 9.



Another colony was cut in two different planes meeting at right angles to each other, (Fig. 10), slicing off a small portion D from the fixed part C. The small piece D contains a number of zooids along the cut edge whose posterior regions have been severed leaving the anterior portions inside the test. This was placed between two small wooden pieces. As in the piece B of the previous experiment, in this fragment D the outer margin of the test

curved inwards due to autolysis and resorption of mutilated zooids, and finally fixed to the substratum assuming a more rounded shape, shown in Fig. 10 D1 and D2. Subsequent growth took place as in all growing colonies, by asexual reproduction.

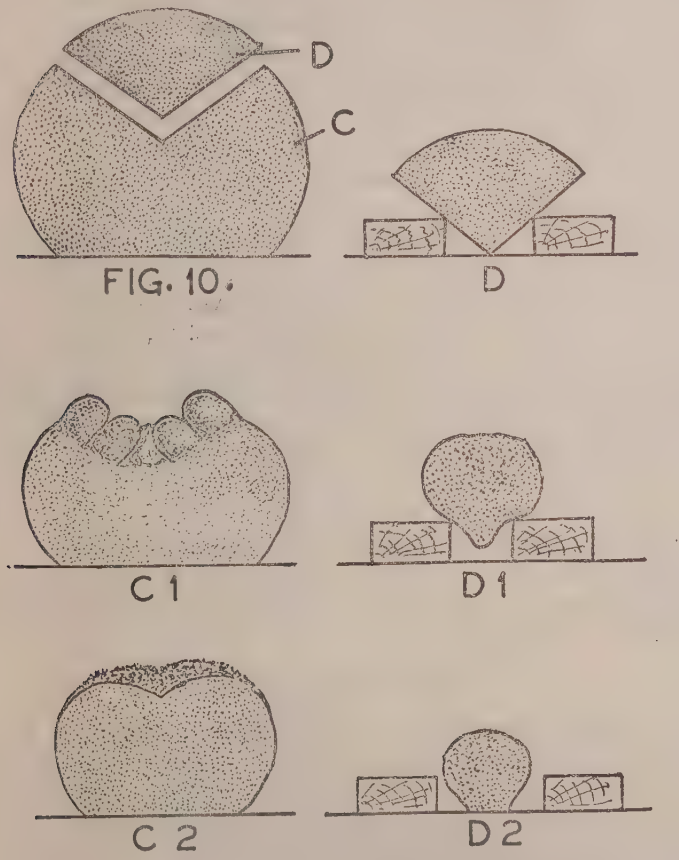


FIG. 10.

The main part C presented the features of regeneration as in the previous experiment. Along the cut edge new zooids were thrust upwards by the secretion of test by the developing buds. More and more zooids were formed after successive asexual reproduction thus filling the gaps on the injured surface. The valley got covered more rapidly than higher up where the cut surfaces diverged and the gulf was wider (Fig. 10 C1), and finally the original shape of the colony was attained (Fig. 10 C2).

Discussion

The results of experiment 1 show that whatever may be the size of a mutilated piece of *Polyclinum indicum*, unless there is a minimum number of zooids inside the test capable of secreting fresh test and undergo asexual budding, such pieces cannot survive. Hence zooids as a group and not as individuals play the part in the survival of the colony. In this connection it is worth recalling the process of restitution after de-differentiation in *P. indicum* described by Sebastian (1954b) wherein the same phenomenon is observed, viz., zooids as a group play the part and not individuals in dedifferentiation of the colony. Another feature met with in both regeneration and dedifferentiation is the role of the test in the upkeep of the colony. If the mutilations sustained by the test is not repaired by rapid secretion of fresh test by the zooids, bacterial action will set in affecting the healthy zooids as well, finally ending in complete decay. Another phenomenon met with in both regeneration and dedifferentiation is the rapidity with which asexual budding takes place in order to overcome the adversity. Only free, healthy buds can help the survival of the colony in both cases. The effort to regain the shape of a mutilated colony is well-marked in *P. indicum* as indicated in experiment 4. Here also the capacity of zooids for asexual reproduction and secretion of test plays the important role. The part played by chemical factors in inducing the survival of the mutilated pieces is evident from experiment 2. What chemical factors other than oxygen play the role is yet to be estimated.

Acknowledgement

This paper formed part of my thesis for the degree of Doctor of Philosophy of the Madras University. I wish to express my thanks to the University of Madras for awarding me a Research Assistantship, and to Dr. C. P. Gnanamuthu, Director, Zoology Research Laboratory, University of Madras, for his help and criticism during the course of the work.

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Nitrogen Balance Studies on South Indian Women

Part I

Effect of variation of calorie intake on nitrogen balance

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ABSTRACT

Nitrogen balance experiments were conducted on four college women (young adults) fed the non-vegetarian hostel diet. In the amounts eaten by these subjects the diet was low in calories. 20 g. sugar along with each of the four meals for each subject provided an additional supply of 320 calories per day. All the subjects excreted less nitrogen in the prine when the diet was made adequate in calories, keeping the protein level the same. The importance of the economic use of dietary protein by attending to the caloric needs of the persons concerned is discussed.

Introduction

Systematic nitrogen balance experiments on adult men in India have been reported by Basu and Basak (1939), Basu, Basak and De (1941), Desikachar *et al.* (1948), Patwardhan *et al.* (1949) and Karambelkar *et al.* (1950) and on adult women by Mason (1934). Mason found the protein intake in the students' hostel diet to be low in both quality and quantity in a small series of nitrogen balance experiments; and that as the calorie intake increased, urinary nitrogen as per cent of intake decreased.

As a continuation of this study by Mason, nitrogen balance experiments were carried out in the same laboratory on volunteers from among the college women living on the hostel diet. As over 80% of the hostel students eat the hostel non-vegetarian diet, this work was confined to a basal non-vegetarian diet. Only after the first two experiments were completed the calorie intake of the subjects was calculated from Tables for food values by Aykroyd *et al.* (1951) (Health Bulletin No. 23) and it was noticed that this diet in the amounts eaten by the subjects was low in calories.

After an interval of nine months, another nitrogen balance experiment was carried out on three of the same student volun-

teers fed the same basal non-vegetarian diet and made adequate in calories by the addition of 80 gms. of sugar (20 gms. with each of the four meals). The present author conducted a separate experiment on herself in which the low calorie diet was followed immediately by the adequate calorie diet for which the same 80 gms. of sugar were used. The purpose of these two experiments was to ascertain the extent to which dietary protein nitrogen would be retained in the body when the diet supplied adequate calories for energy expenditure for the moderately active college woman.

Experimental

Preliminary survey of the hostel diet

A rough survey of the hostel non-vegetarian diet was carried out for a week, since a weekly dietary regime is followed in planning the meals. Then a more precise study was made on the diet of four students who kept a record of the actual amounts (in g.) of prepared foods eaten by them at each meal for one week. For each student a fourth of the quantities of the food preparations eaten each day were weighed and stored in the refrigerator for the analysis of total nitrogen. From these analyses the average daily nitrogen intake was estimated. This ranged from 5·33 to 7·22 g. representing 33·3 g. to 45·1 g. protein. The average maximum quantity of protein ingested by any student who ate all that was given in the hostel diet was 45 gms. while planning the diet for nitrogen balance experiments, the daily protein intake was kept close to this maximal level.

Plan of Experiments

The subjects were carefully chosen after personal interviews from among the students taking courses in physiology and nutrition hence they understood the significance of the experiment. The experiment was conducted on three students at a time, fed the basal non-vegetarian diet under the supervision of the investigator and her assistants. The volunteers ate all their meals in the laboratory where they were prepared and served.

Four nitrogen balance experiments were carried out. In three of these experiments, the duration of the experiment was 12 days and menu was for a 3 a day period (Leverton & Gram 1949) which was repeated four times. The diet was planned to resemble closely the students' non-vegetarian hostel diet in quality, quantity and distribution between the four meals. Cooked rice was served at two main meals at noon and night and the animal protein foods

TABLE 1

The consumption on a three-day dietary pattern — g./day

(Cooked foods)

	1st Day					2nd Day					3rd Day				
	ME	KJ	MKJ	AJ	AA	ME	KJ	MKJ	AJ	AA	ME	KJ	MKJ	AJ	AA
Breakfast 8 a.m.															
Sugar	10	9	12	9	18	10	9	12	9	18	10	9	12	9	18
Condensed milk	6	10	10	10	10	6	10	10	10	10	6	10	10	10	10
Puri	85	80	87	80	80	78	75	78	90	90	85	80	87	80	80
Potato	93	88	92	80	115	60	64	60	64	64	93	88	92	80	115
Plantain	40	50	40	50	50	42	35	42	35	35	40	50	40	50	50
						40	50	40	50	50					
Lunch 12 noon															
Rice	230	285	240	205	275	230	285	240	205	275	230	285	240	205	215
Sambar	69	58	68	53	74	69	58	68	53	74	69	58	68	53	74
Brinjal	30	20	27	14	20										
Greens	30	25	30	25	25										
Fish fried	38	35	32	35	35										
Curds	90	80	90	80	80	90	80	90	80	80	90	80	90	80	80
Drumstick						22	11·5	14	12·5	7·2					
Carrot salad						25	25	25	25	25					
Egg omlette						35	35	35	35	35					
Onion											10	10	10	17	16
Beans											30	30	30	30	30
Carrot											25	25	25	25	25
Mutton											35	25	24	25	25
Tea 4 P.M.															
Vadai	57	70	57	70	70										
Bonda											80	80	80	80	80
Bread						38	40	38	40	40					
Butter						5	5	5	5	5					
Puff						44	50	37	50	50					
Condensed milk	—	10	—	10	10	—	10	—	10	10	—	10	—	10	10
Plantain	40	50	40	50	50	40	50	40	50	50	40	50	40	50	50
Sugar	19	18	18	18	21½	19	18	18	18	21½	19	18	18	18	21½
Dinner 7-30 P.M.															
Rice	220	200	240	200	200	220	200	240	200	200	220	200	240	200	200
Sauce	69	56	68	43	53	69	56	32	43	53	69	56	68	43	53
Mutton	26	25	27	25	25										
Potato	33	23	33	23	23	26	25	24	25	25					
Tomato salad	40	30	40	30	30						40	30	40	30	30
Onion salad	7	5	7	5	5						7	5	7	5	5
Beans	30	30	30	30	30										
Brinjal						30	20	27	14	20					
Greens						30	25	30	25	25					
Fish											30	35	35	35	35
Banana chips											10	10	10	10	10

were confined to these meals. The menus and the composition of the diet are shown in Table 1. Two 3-day collections of food, urine and feces were made during the latter half of the experimental period.

The experiment was repeated on the same subjects after an interval of nine months, when 80 gms. of sugar was given additionally to each subject. This was divided equally and given with each of the four meals, since Cuthbertson and Munro (1939), Geiger (1951) and others have stressed the importance of feeding the carbohydrate and protein moieties of the diet together.

Preparation and serving of food.

Each dish was prepared in one lot for the three subjects, keeping the recipe the same for the entire period of the experiment. Individual servings which were weighed represented what each person was accustomed to eat in the hostel.

Collection, sampling and analysis for total N_2

During the balance period (the second six days) cooked rice, puri, plantain and condensed milk were analysed separately. Other dishes were grouped together according to whether or not the individual servings were of the same weight for all the subjects. For each three day period, the dishes that weighed the same for all, were collected in one lot in duplicate, labelled as 'constant factor' and stored in the refrigerator. Those dishes for which the weights were different for the different individuals, were weighed separately for each person for the two three day periods, collected and stored as the variable factor. In one experiment, the foods eaten at tea time were put together in one lot as the tea factor. In another experiment in order to find out the proportion of the animal protein to the total protein intake per day, the animal protein foods were collected in one lot for each three day period.

Food samples thus collected for two three day periods were homogenized in the waring blendor and the total weight of the slurry noted for each sample. 5 to 10 g. portions of the slurry were weighed accurately in weighing bottles and transferred quantitatively into kjeldahl flasks for Macro-kjeldahl nitrogen estimation in triplicate. Brickner *et al.* (1945), Hegsted *et al.* (1946) and Leverton and Gram (1949) and others have used this technique for sampling cooked foods for nitrogen estimation.

In the experiment the author had performed on herself the diet was planned for one day and the same menus were repeated

for 9 days on the low calorie diet. The subject immediately changed over to the adequate calorie diet for 9 days (the diet made adequate in calories by adding 80 g. sugar). Both Hegsted (*loc. cit.*) and Patwardhan (*loc. cit.*) have used this single day regime which was better controlled than the one mentioned earlier. As Hegsted (*loc. cit.*) and others found a period of three days too short for the subject to adjust to a dietary pattern and attain equilibrium, the first five days of each 9-day period were reckoned as the period of adjustment to the test diet and the following four days as the balance period when food, urine and feces were collected for analysis of total nitrogen. For all subsequent experiments this plan was followed. The menus and the composition of the diet are given in Table 2.

TABLE 2

Amounts of uncooked foods eaten/day for F.T.
(Daily dietary regime)
E.P. = Edible Portion

Foodstuffs used in the diet	E.P. wt. g.	Foodstuffs used in the diet	E.P. wt. g.
Breakfast		Dinner	
Wheat flour whole	.. 50	Rice—parboiled milled	.. 75
Potato	.. 45	Fish	.. 30
Dalda	.. 12·5	Beans	.. 20
Onions	.. 12	Brinjal	.. 20
Sugar	.. 8	Coconut	.. 13
Green chillies	.. 1	Coriander	.. 1·5
Plantain	.. 50	Green chillies	.. 0·5
Condensed milk	.. 5	Dalda	.. 6
Lunch		Chilli powder	.. 1
Rice—parboiled milled	.. 75	Menus	
Dhal arhar	.. 20	Breakfast—Puri, potato and plantain.	
Mutton	.. 30		
Amaranth	.. 20	Lunch — Rice, sambar (dhal) greens, mutton and potato fried.	
Drumstick	.. 18		
Potato	.. 15	Tea — Vadai, tea and plantain.	
Onion	.. 10	Dinner — Rice, fish sauce, brinjal and beans.	
Green chillies	.. 0·5		
Coconut	.. 3		
Dalda	.. 8		
Chilli powder	.. 1		
Tea			
Black gram dhal	.. 20		
Condensed milk	.. 5		
Sugar	.. 8		
Green chillies	.. 1		
Onions	.. 7		
Dalda	.. 6		
Plantain	.. 50		

TABLE 3

Data on nitrogen intake — average for two 3-day dietary period and the mean daily nitrogen intake
L.C.C. = Low calorie diet, A.C.D. = Adequate calorie diet

Food Samples	ME	KJ	MKJ		AJ		AA	
	L.C.D. g.	L.C.D. g.	L.C.D. g.	A.C.D. g.	L.C.D. g.	A.C.D. g.	L.C.D. g.	A.C.D. g.
Rice — cooked ..	4·74	4·08	4·71	4·74	3·49	3·49	4·24	4·24
Puri ..	2·35	1·96	2·40	2·35	1·96	1·96	1·96	1·96
Bread ..	1·38	1·30	1·38	1·38	1·48	1·48	1·48	1·48
Plantain ..	0·12	0·54	0·12	0·12	0·54	0·54	0·54	0·54
Condensed milk ..	0·08	0·83	0·42	0·83	0·83	0·83	0·83	0·83
Other foods ..	13·69	13·96	13·38	13·02	13·43	12·74	13·28	12·94
Dry milk powder ..	1·71	—	—	—	—	—	—	—
Total N_2 intake for 3 days ..	24·07	21·67	22·41	21·99	20·73	21·04	22·33	21·99
Mean daily intake ..	8·02	7·22	7·47	7·33	6·91	7·01	7·44	7·33

Preparation and serving of food

The food preparations on the menu for each of the four meals per day were cooked in individual servings for each student from weighed quantities of uncooked foodstuffs included in the recipes. This method involved a great deal of work, but this was more accurate than the previous one. The food was prepared and served in the laboratory and eaten in the laboratory under supervision.

Sampling and analysis for total nitrogen.

Identical meals were prepared during the balance period (two 2-day collections) and stored for analysis. Cooked rice, puri, bread, curds and mutton preparations were analysed separately. The method of sampling and analysis was same as above.

Collection and analysis of urine and feces

On the three day regime, urine was collected for the third and fourth three day periods. 24 hours collections were timed from the first collection in the morning after evacuating the bladder of the night urine. Each days urine was collected under toluene, measured and transferred into a storage bottle containing 5 ml. 2N Hcl (Cullumbine, 1951) for each three day period. 5 ml. samples of urine were pipetted out (after separating the toluene) in kjeldahl flasks for N_2 estimation in triplicate.

TABLE 4

Data on nitrogen intake for subject F.T. on the single day dietary regime—average for two 2-day collection periods and the mean daily nitrogen intake.

Food Samples.		Low calorie diet g.	Adequate calorie diet g.
Rice cooked	..	3·58	3·58
Puri	..	1·56	1·56
Plantain	..	0·36	0·36
Condensed milk	..	0·55	0·55
Other foods	..	8·66	9·23
Total nitrogen intake for two days	..	14·71	15·28
Mean daily intake	..	7·36	7·64

On the single day dietary plan, urine was collected separately for four days. Two days collections were combined for analysis.

TABLE

Data on N_2 Balance Experiments on

Duration of Expt. days.	Balance period days.	Subject.	Body weight kg.	Average cals/day.	Calories/kg. body wt.	Total protein intake/day g.
		No.				
12	6	3. M.K.J.	40·45	1509	37·30	51·25
12	6	2. K.J.	35·00	1726	49·30	45·19
12	6	1. M.E.	46·36	1573	33·92	46·69
12	6	4. A.J.	46·82	1555	33·21	43·19
12	6	5. A.A.	56·82	1788	31·47	46·57
9	4	6. F.T.	44·55	1581	35·49	46·01
				Data on N_2 Balance Experiments on		
12	6	3. M.K.J.	46·36	1893	40·83	46·76
12	6	4. A.J.	47·04	1875	39·95	44·07
12	6	5. A.A.	56·36	2100	37·26	45·81
9	4	6. F.T.	45·00	1901	42·25	47·75

llege Women — Low Calorie Diet

Protein intake g./kg. body wt.	N_2 intake per day g.	Urinary N_2/day g.	Faecal N_2/day g.	Balance.	Urinary N_2 / Intake N_2 %	Faecal N_2 / Intake N_2	Retention %
1·27	8·02	5·62	1·43	+0·97	68·53	17·44	11·83
1·29	7·23	5·83	1·41	−0·01	82·52	19·50	−0·15
1·01	7·47	5·25	1·15	+1·07	70·30	15·39	14·32
0·92	6·91	5·50	1·32	+0·09	79·60	19·10	1·30
0·80	7·45	5·10	1·89	+0·36	68·45	25·36	4·83
1·03	7·36	5·05	1·49	+0·82	68·61	20·24	11·14
ollege Women — Adequate calorie diet.							
1·01	7·48	4·56	1·16	+1·76	59·58	15·51	23·53
0·94	7·05	4·20	1·27	+1·58	59·57	18·01	22·42
0·81	7·33	4·59	1·33	+1·41	63·01	18·14	19·23
1·13	7·64	4·31	1·81	+1·52	56·41	23·69	19·89

CORRECTION : pp. 500-501 - Table 5.

The following is the correct order of subjects for the data given in this table for the Low Calorie Diet:

1. ME
2. KJ
3. MKJ
4. AJ
5. AA
6. FT

Feces marked with carmine (0·3 g. in gelatin capsule No. ·00) were collected in porcelain dishes, weighed immediately and transferred quantitatively into a screw top glass storage bottle containing 400 cc of 2N HCl for each three day period. On the single day regime, feces were collected in the same bottle for the entire balance period of four days. As suggested by Hawk, Oser and Summerson (1954) N_2 analysis was carried out on the *wet* acidified samples in order to prevent deterioration during drying. The acidified feces were homogenized in the waring blendor, the total weight of the slurry noted and 10 to 15 g. portions of the slurry were weighed accurately and transferred quantitatively into kjeldahl flaks for N_2 estimation in triplicate.

Results and Discussion

Data on nitrogen intake for the subjects on the three day dietary regime are given in Table 3, the one subject on the single day regime are given in Table 4. Subjects MKJ, AJ, AA & FT were on both the low calorie diet and on the adequate calorie diet. Complete data on nitrogen balance are presented in Table 5.

For the six subjects on the basal diet the average daily nitrogen intake varied from 7 to 8 g. and the average daily urinary nitrogen excretion varied from 5·05 to 5·83 g. Four subjects (3, 4, 5 & 6) who were on both diets excreted less nitrogen in the urine when the caloric value of the diet was made adequate by the addition of 80 g. cane sugar per day which furnished 320 calories more per person per day. The daily urinary nitrogen excretion for these four subjects on the adequate caloric diet ranged from 4·20 to 4·59 g. and for the same subjects on the low calorie diet, the urinary nitrogen excretion varied from 5·05 to 5·50 g. Thus the addition of 320 calories prevented the loss of about 1 g. nitrogen in the urine.

Leverton *et al.* (1951) have reported a study of the effect of caloric level on nitrogen utilization of young women. When eight women 17 to 20 years of age and of body weight 100 to 150 lbs. were on a diet which supplied 43 g. of protein and 1800 cals/day (period A), the mean daily urinary nitrogen excretion exceeded nitrogen intake and retention calculated as per cent of intake was $-9{\cdot}3\%$. When the energy value of the diet was changed to 2400 calories (period B) with the same level of protein intake, the same eight women showed an average retention of $+8{\cdot}4\%$. Leverton *et al* (*loc. cit*) reported that this increase in retention was due to the highly significant decrease in the mean daily urinary nitrogen from 6·77 g. in period A to 5·56 g. in period B.

At a higher level of protein intake (63 g.) also, an increase in retention was observed in period B when the calories were increased to 2400. Thus increasing the calories from 1800 to 2400 had a sparing action on nitrogen metabolism at both levels of protein intake but the action was more significant at the lower level.

Mason (1934) showed that on the student diet three students excreted in the urine, 88% of intake nitrogen when the calorie content of the diet was only 1300; where as on an isoprotein experimental diet with a calorie content of over 2000, they excreted only 41% of intake nitrogen. Munro (1951) has reviewed in detail the investigations on the relationship of nitrogen balance to energy intake and the adverse effect of withdrawal of calories on nitrogen balance. In the present investigation the caloric value of the breakfast meal was quite low and the students did feel hungry an hour before lunch time when they were on the low calorie diet. They were quite satisfied with the meals when the calorie deficit was made good.

According to a public health report by Drummond (1952) a mean figure of 1500 calories per person per day is a critical level. Keys (1949) has pointed out that the body size and activity should be taken into account in estimating calorie requirements of adults. He refers to 3000 calories for the physically active 70 kilogram man per day as an adequate level. This works out to 43 cals/kg. body weight per day, which is close to 40 cals/kg. body weight/day suggested by Sherman (1920) for the moderately active man and woman. In the present investigation on the adequate calorie diet the calorie intake was at the 40 calorie level for three subjects where as on the low calorie diet the range of calorie intake for all the four subjects was from 32 to 36 cals/kg. body weight.

Even though the relation of energy intake to nitrogen utilization has been known for many years, in India where the protein foods particularly the animal protein foods are expensive and where many hostels have limited money for food, it is very important that the protein should be utilized economically to the greatest advantage for the anabolic process and the energy value of the diet should meet the person's energy requirement.

Summary and Conclusion

Nitrogen balance experiments were carried out on four college women who ate a basal non-vegetarian diet planned to resemble

the hostel non-vegetarian diet. It was found that for all subjects this diet (in the amounts eaten by them) was low in calories.

For the same subjects when the calorie intake was made adequate the urinary nitrogen excretion was less than when the calorie intake was inadequate. Hence it is concluded that dietary nitrogen will not be utilized economically if the energy value of the diet does not meet a person's energy requirement.

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Nitrogen Balance Studies on South Indian Women

Part II

Effects on the Quality of Dietary Protein on Nitrogen Balance.

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ABSTRACT

The quality of the dietary protein in a non-vegetarian basal diet was compared with that of an experimental vegetarian diet, by conducting nitrogen balance experiments on college women. One third of the protein in the basal diet was derived from animal foods. The two diets were isoprotein and isocaloric. On the basal non-vegetarian diet, the subjects excreted more nitrogen in the urine than on the experimental vegetarian diet which consisted mainly of cereals and pulses and practically no animal protein foods. The above results are discussed in relation to the Essential amino-acid composition of the two diets and are compared with results of other workers.

Introduction

In 1897 Rubner recognized the proteins from different sources were not of the same nutritive value. Osborne (1907) pointed out the deficiency of lysine in the proteins of the wheat kernel. The superiority of casein over zein was also observed. Zein was found to be deficient in the essential amino acid-tryptophane. As more and more differences between animal and vegetable proteins were brought to light, animal proteins came to be classified as superior to vegetable proteins.

Within the past decade a great deal of work has been done both in India and abroad in studying the nutritive value of vegetable protein diets and mixed diets which included different proportions of animal protein in the total dietary protein. Studies in mutual supplementation of vegetable protein foods other than cereals and pulses were carried out by Lal & Rajagopal (1953). Patwardhan and his associates (1949) and Karambelkar and his co-workers (1950) have studied the influence of dietary nitrogen

on urinary nitrogen excretion. Their basal diet was predominantly vegetarian diet and when one-third of the vegetable protein was replaced by animal protein as milk, egg or mutton, the urinary nitrogen excretion increased. The subjects were still in positive nitrogen balance but the retention was much less here than on the predominantly vegetarian diet.

In the basal non-vegetarian diet referred to, in the nitrogen balance studies on South Indian women — Part I, animal protein constituted about one-third of the total dietary protein. In the experiments which will be reported in this paper, this diet was compared with an experimental vegetarian diet in which pulses replaced the animal protein foods.

Out of the hostel diet surveyed in twenty different schools in South India (Theophilus 1946), it was found that animal protein was less than 5% of the total protein intake in 14 hostels (0·1% to 3·8 %), less than 10% in 5 hostels and 14% in one hostel. In many of these diets there was practically no milk and the animal protein food, chiefly mutton, only once or twice in a week in one meal. Cereals and pulses formed the bulk of the diet. It was felt that useful information might be obtained regarding the nutritive value of predominantly vegetable protein diets by nitrogen balance experiments carried out on college women fed first the basal non-vegetarian diet followed immediately by an experimental vegetarian diet.

Experimental

The experiment was carried out for eighteen days on four college women—three students (18-20 yrs. old) and the investigator (over 30 yrs. old). The subjects were fed the basal non-vegetarian diet for the first 9 days and immediately following this period they were fed the all-vegetarian experimental diet for 9 days. In each nine day period, the first five days represented the period of adjustment to the test diet and next four days, the balance period when the nitrogen intake and output were measured. The daily dietary regime described in Part 1 was followed here. The experimental technique and chemical analysis were also the same.

When the animal protein foods were replaced by vegetable protein foods, care was taken to keep the calorie content and quantity of protein the same in the two diets. Adjustments were not made with regard to other nutrients. The menus for the two diets are as follows :—

Meal.	Non-vegetarian basal diet.	Experimental vegetarian diet.
	Coffee at 6-45 A.M.	
	Breakfast — 8 A.M. Puri, potato and plantain.	
Lunch—12 noon.	Rice, drumstick and dhal sambar, greens, mutton & potato fried, curds.	Rice, drumstick and dhal sambar, greens, whole bengal gram and potato fried.
	Tea—4 p.m. Vadai, bread butter, plantain and tea.	
Dinner—7-30 p.m.	Rice, brinjal and mutton curry, beans.	Rice, brinjal and dhal curry and beans.

The amounts of foods (uncooked) used in the daily meals are also given (Table 1).

Complete data on nitrogen balance for the four day collection period are available for two students and the investigator. For the third student, the collection of urine was for two days only after the five-day adjustment period. The students ate all their meals in the laboratory under supervision.

Results and Discussion

Analysis of total nitrogen in food and excreta are given below (Table 2). For subject F.T., results are given for two nitrogen balance experiments at two levels of animal protein (13% and 33%) in the non-vegetarian basal diet. At 13% level, there was practically no difference in the level of urinary nitrogen excretion in the animal and vegetable proteins periods, but at the 33% level, a decrease in the urinary nitrogen excretion was noted when the subject changed from the animal protein to the vegetable protein diet. In both the balance experiments there is a slight increase in the elimination of nitrogen in the feces during the vegetable protein period.

Subject M. K. J., maintained a very good balance in both periods. She was at a slightly higher level of protein intake as she took "extra", milk along with the basal diet. The mean daily nitrogen intake for this subject was slightly more than one gram/kilogram body weight and the animal protein formed 38% of the total protein. On the basal diet she eliminated in the urine 80% of the total nitrogen intake per day and on the vegetable protein diet 78%. The difference is very little. She eliminated 20%

TABLE 1

Amounts of foodstuffs in g. (uncooked) included in the daily menus of the two diets.

Foodstuffs.	MKJ		MG		ML		FT (I)		FT (II)	
	Non-veg.	veg.	Non-veg.	veg.	Non-veg.	veg.	Non-veg.	veg.	Non-veg. 13% protein level.	veg.
Cereals										
Rice (Parboiled, milled) ..	150	150	120	120	130	130	120	120	150	150
Wheat flour ..	50	65	50	50	50	65	50	50	50	50
Bread ..	25	16	25	12	25	20	25	25	—	—
Pulses										
Dhal arhar ..	20	20	20	20	20	20	20	20	48	72
Black gram dhal ..	20	55	20	35	20	45	20	30	30	30
Green gram dhal ..	—	20	—	20	—	20	—	23·5	—	—
Whole Bengal gram ..	—	30	—	30	—	30	—	37	—	—
Vegetables										
Potato (Boiled) ..	60	60	60	60	60	60	60	60	60	60
Drumstick pod ..	20	20	20	20	20	20	20	20	20	20
Amaranth ..	20	20	20	20	20	20	20	20	20	20
Brinjal ..	20	20	20	20	20	20	20	20	20	20
Beans ..	20	20	20	20	20	20	20	20	20	20
Condiments										
Cocoanut ..	16	3	16	3	16	3	16	3	16	16

Onions	..	36	40	36	36	36	38	36	36	38	38
Green chilli	..	2·7	3	2·7	2	2·7	2·8	2	2	2·7	2·7
Tamarind	..	3·0	3	3·0	3	3	3·0	3	3	2·5	2·5
Chilli powder	..	1·5	1·5	1·5	1·5	1·5	1·5	1	1	1	1
Fats and Oils											
Butter	..	5·0	5·0	5·0	5·0	5·0	5·0	5·0	5·0	—	—
Dalda	..	43·42	50·88	43·42	43·29	43·42	40·98	43·42	47·42	39	39
Milk and milk products											
Milk (whole)	..	210	—	—	—	145	—	—	—	—	—
Condensed milk	..	5	5	5	5	5	5	5	5	10	10
Curds	..	80	—	80	—	80	—	80	—	—	—
Fleshy foods											
Mutton	..	60	—	60	—	60	—	60	—	30	—
Fruits											
Plantain	..	100	100	100	100	100	100	100	100	100	100
Sugar	..	55	40	45	30	30	20	70	39	78	78
'Chutney'	..	14	6	14	14	14	14	6	6	—	—
Calculated from Tables of Food Values. (Aykroyd et al. 1951).											
Total Calories.	..	2246	2169	2066	1975	2035	2017	2008	2010	1940	1940
Protein g.	..	54·66	52·34	46·15	44·18	51·24	49·71	45·01	45·01	45·01	44·99
Carbohydrate g.	..	317·90	376·28	299·01	296·02	274·15	305·48	305·31	305·14	334·41	338·14
Fat g.	..	85·42	66·72	77·66	63·55	82·92	67·81	68·66	88·41	53·54	45·96

TABLE 2.

Data on Nitrogen Balance Experiments on College Women.

Subject	Diet	Body wt. kg.	Calories per day	Calories per kg. body wt.	Average Total protein g./day	Protein per kgm. body wt.	Animal protein av./day	Total N_2 intake g. per day	Animal protein N_2 g./day
I. F.T.	Basal diet—A.Pr. ..	44·55	1940	43·55	47·03	1·06	5·30	7·52	0·85
	Veg. Pr. Diet ..	44·55	1940	43·55	47·21	1·06	0·88	7·55	0·14
II. F.T.	Basal—A.Pr. ..	44·55	2008	45·07	43·37	0·98	14·22	6·95	2·28
	Veg. Pr. Diet ..	44·55	2010	45·12	42·06	0·94	0·44	6·73	0·07
M.K.J.	Basal diet—A.Pr. ..	47·39	2246	47·39	55·23	1·17	21·34	8·88	3·41
	Veg. Pr. Diet ..	47·27	2169	45·90	52·70	1·11	0·44	8·43	0·07
M.L.	Basal—A.Pr. ..	42·73	2035	47·62	52·16	1·22	19·32	8·35	3·09
	Veg. Pr. Diet ..	43·43	2017	46·44	49·93	1·15	0·44	7·99	0·07
M.G.	Basal—A.Pr. ..	46·02	2066	44·85	43·95	0·95	14·22	7·03	2·28
	Veg. Pr. Diet ..	46·20	1975	42·71	42·19	0·91	0·44	6·75	0·07

Subject	Diet	A. Pr. N_2% Total protein N_2	Av. urine N_2 per day g.	Faecal N_2 per day g.	Balance	Urine N_2 Intake N_2%	Faecal N_2 Intake N_2%	Retention %	Urine N_2 kgm. body wt. mgms.
I. F.T.	Basal diet—A.Pr. ..	11·30	4·06	1·76	+1·70	54·01	23·44	22·54	91·2
	Veg. Pr. Diet ..	1·85	3·92	2·05	+1·59	51·87	27·11	21·01	87·9
II. F.T.	Basal—A.Pr. ..	32·75	4·88	1·73	+0·34	70·31	24·85	4·80	109·6
	Veg. Pr. Diet ..	1·06	4·17	2·04	+0·52	61·99	30·33	7·69	93·6
M.K.J.	Basal—A.Pr. ..	38·43	7·11	1·78	—0·01	80·05	20·04	—0·01	150·1
	Veg. Pr. Diet ..	0·84	6·60	1·76	+0·07	78·32	20·87	+0·80	139·7
M.L.	Basal—A.Pr. ..	37·02	5·81	1·48	+1·06	69·58	17·72	12·70	136·0
	Veg. Pr. Diet ..	0·89	5·22	1·79	+0·98	65·33	22·40	12·27	120·2
M.G.	Basal—A.Pr. ..	32·35	5·08*	1·31	+0·64	72·23	18·63	9·15	110·4
	Veg. Pr. Diet ..	1·05	4·75*	2·09	—0·09	70·37	30·98	—1·35	102·8

*Average of two-day collections only.

nitrogen in the feces on both diets. There was practically no retention of nitrogen in either diet.

For subject M.L., animal protein formed 37% of the total protein and the mean daily intake was over one gram per kilogram body weight. On the basal diet she eliminated about 70% of the total nitrogen intake per day and on the vegetable protein diet 65%. The difference is not striking.

For subject M.G. also the urinary nitrogen excretion was slightly less on the vegetable protein diet than on the non-vegetarian basal diet.

The significance of the difference between the utilization of protein in a practically all-vegetarian diet and in the animal protein diet can only be studied in adults at the maintenance level. As Hegsted (1952) points out, the body utilizes nutrients less efficiently as the intake rises. When the intake falls, there might be a temporary loss of stored nutrients and provided the level is above the minimum requirement, the body would soon strike a new balance. Mitchell (1923) has shown that the biological value of proteins is less at high levels of protein intake than at the lower level.

In the present investigation, for subject M. K. J., on an intake of 1 g. protein/day/kg. body weight (recommended allowance) almost all the nitrogen absorbed from the gut was eliminated in the urine. Data on a number of human subjects at different levels of protein intake will throw light on the level at which the proteins could be used economically. This will be a valuable study in India where protein foods in general are too expensive for the lower income groups.

The experiments reported here on nitrogen balance with the basal non-vegetarian and the all vegetarian diets are in some ways different from the experiments reported by Patwardhan *et al.* (*loc cit.*) and Karembelkar *et al.* (*loc cit.*). The basal diet used by these workers was a predominantly vegetarian diet, as their volunteers were habitual vegetarians whereas in the present investigation, the subjects were habitual non-vegetarians and in the basal diet 30% of the total protein was from animal origin. In the predominantly vegetarian diet Patwardhan *et al.* (*loc. cit.*) and Karambelkar *et al.* (*loc. cit.*) the quantity of animal protein varied from 4·7% to 16·1% and from 7·2% to 22·5% respectively. In the present investigation in the experimental vegetarian diet, the

animal protein formed 0·84% to 2% of the total protein intake per day. In the animal protein diet of Patwardhan *et al.* (*loc. cit.*) 50% of the total protein was replaced by animal protein (Diet 11) for the three subjects. In the animal protein diet of Karambelkar *et al* (*loc. cit.*) the animal protein formed 41·7% to 47·9% of the total protein. In the present investigation animal protein formed 32% to 38% of the total protein.

In the present investigation (as reported already) on the vegetable protein diet, urinary nitrogen excretion was less than on the non-vegetarian basal diet; but this difference is not so striking for the subjects in the present investigation as for all the volunteers of Patwardhan *et at.* (*loc. cit.*) and for volunteers Nos. 1, 3, 4 and 6 of Karambelkar *et al.* The subjects in the present investigation behave very much like subjects Nos. 2, 5 and 7 of Karambelkar *et al.* (*loc. cit.*) as may be seen from the data on the urinary nitrogen excretion as per cent of nitrogen intake, given below for these three subjects and for four subjects of this investigation.

Urinary nitrogen as % of nitrogen intake.

		Basal vegetarian diet %	Experimental animal protein diet %
Karambelkar et al (loc. cit)	Subject 2 ..	67·7	73·2
	Subject 5 ..	67·3	69·8
	Subject 7 ..	55·4	58·5
		Experimental vegetarian diet	**Basal non-vegetarian diet**
Present investigation	Subject 1 ..	62·0	70·3
	Subject 2 ..	78·3	80·1
	Subject 3 ..	65·4	69·6
	Subject 4 ..	70·4	72·3

It has been shown from nitrogen balance experiments on adult human subjects in the U.S.A. that the urinary nitrogen excretion is less on an animal protein diet than on a vegetable protein diet. Murlin *et al.* (1938), Hegsted *et. al.* (1946). This is contrary to the results obtained on Indian diets. It should be pointed out that the experimental vegetable protein diets of Murlin (*loc. cit.*)

Basal All vegetarian diet (Hegsted et al.)		Basal predominantly vegetarian diet (Patwardhan et al.) Subject 1.		Experimental vegetarian diet (Present investigation) Subject F.T.	
	g		g		g
Bread (no milk solids)	.. 144	Whole wheat flour	.. 336	Bread	.. 25
Rice	.. 24	Rice	.. 114	Rice (parboiled milled)	.. 120
Yellow corn meal	.. 12	Pulses	.. 71	Whole wheat flour	.. 50
Potatoes	.. 160	Other legumes	.. 21	Pulses and legumes	.. 111
Onions	.. 40	Vegetables and spices	.. 227	Potato	.. 60
Carrots (canned)	.. 160	Fruits	.. 71	Amaranth leaves	.. 20
Tomatoes (canned)	.. 80	Sugar and jaggery	.. 71	Other vegetables	.. 60
Lettuce	.. 80	Oil and ghee (butter fat)	.. 71	Onions	.. 26
Apple sauce (canned)	.. 80	Milk	.. 227	Coconut	.. 16
Orange juice	.. 160			Plantain fruit	.. 100
Peaches (canned)	.. 80			Sugar	.. 39
				Oil and butter	.. 52
				Condensed milk	.. 5
Cereal and cereal products	.. 168g.	Cereal and cereal products	.. 450g.	Cereal and cereal products	.. 195g.
Pulses and other legumes	.. Nil	Pulses and other legumes	.. 92g.	Pulses and other legumes	.. 111g.
			542g.		306g.
Potato	.. 160g.	Cereals 83%	(5/6th)	Cereals 64%	(2/3)
		Pulses 17%	(1/6th)	Pulses 36%	(1/3)

and Hegsted (*loc. cit.*) consist chiefly of cereals and vegetables and have no pulses or other legumes whereas the Indian vegetarian diets consist of a considerable amount of pulses in addition to a large amount of cereals. The composition of vegetarian diets used by Hegsted *et al.* (*loc. cit.*) Patwardhan *et al.* (*loc. cit.*) and the present investigator are given above:

Phansalkar and Patwardhan (1956) in their recent publication have pointed out two reasons for the difference in results obtained by the American and Indian workers while comparing a predominantly animal protein diet with a vegetable protein diet.

One reason is that the level of protein intake was minimal for the American subjects. The second is that as seen in the above table for Hegsted's diet as well as for diets used by Murlin (*loc. cit.*) the vegetable protein diet was comprised of cereal protein, whereas in Indian dietaries the vegetable protein was derived from a mixture of cereals and pulses.

Recently Vijayaraghavan and Srinivasan (1953) have determined the essential amino acid content of some of the common pulses and lentils and have shown them to be rich in lysine which is the limiting essential amino acid in cereals. The two types of food, (cereals and pulses) complement each other with regard to this amino acid.

Rose (1950) has given figures for the minimum requirement of eight essential amino acids for man, the recommended allowance being reckoned as double the minimum requirement. The essential amino acid compositions of the two test diets (used in the present investigation) were calculated from the tables of essential amino acid composition of food stuffs published by Block and Bolling (1951) and Vijayaraghavan and Srinivasan (*loc. cit.*). The figures presented in the table (3) below are in terms of an adult weighing 70 kilograms since Rose's figures are for this body weight. It may be gathered from these data that even the vegetable protein diet has more than satisfied the recommended allowances of six of the essential amino acids and the minimum requirement of the other two. In the non-vegetarian diet the essential amino acids found over and above the level of these in the vegetable protein diet are probably deaminized and excreted in the urine. It may be pointed out that there is very little difference in the essential amino acid content of these two diets. Only with regard to tryptophane and methionine the difference is considerable.

TABLE 3

The essential amino acid content of vegetable and animal protein diets for subject F.T.* (In one day's protein intake).

Essential amino acid.	Vegetable protein diet.	Animal protein diet.	Rose's minimum requirement per day.	Rose's recommended allowance/day.
Isoleucine ..	3·37	3·05	0·7	1·4
Leucine ..	4·70	5·10	1·1	2·2
Lysine ..	3·49	3·98	0·8	1·6
Methionine ..	1·01	1·41	1·1	2·2
Cystine ..	0·71	1·21	—	—
Phenylalanine ..	3·75	3·62	1·1	2·2
Threonine ..	2·55	2·70	0·5	1·0
Tryptophane ..	0·46	0·67	0·25	0·5
Valine ..	3·56	3·27	0·8	1·6

* Calculated for 70 kg. body weight.

Hegsted *et. al.* (1946) have shown that for a diet in which a third of the protein was from animal origin the total protein intake can be less by 17% than when the diet derives all its protein entirely from vegetable sources. This marked difference was because Hegsted's vegetarian diet lacked pulses. Brickner *et al.* (1945) have carried out nitrogen balance experiments on adult college women to study protein requirement in terms of the protein contained in individual foods and food combinations. They have found that the average daily requirement of conventional protein ($N \times 6{\cdot}25$) during adult life in terms of a 70 kg. man are: 43 g. of milk, 74 g. for white flour and 47 g. for soya flour. From this study as well as from Hegsted's data, it appears that the higher the average quality of protein in the diet the less the amount needed to provide for maintenance, adult growth and functions in the adult.

Lysine is the limiting amino acid in cereal protein, hence a much greater amount of white flour protein is required (if white flour is to provide all the protein for the day) to raise the lysine content of the protein to the maintenance level. If for instance 74 g. of protein is supplied both in the white flour diet and the milk

diet, the essential amino acids in the milk diet will be in greater concentration and the surplus amino acids will simply be deaminized and excreted in the urine. Phansalkar and Patwardhan (1954) found that when their adult men were on animal protein diet, urea nitrogen excretion in the urine was greater than when they were on isoprotein adequate predominantly vegetable protein diet. Here also the surplus amino acids in the animal protein diet probably account for greater urinary nitrogen excretion. With a better quality of dietary protein, the quantity might be reduced. The recommended allowances for protein intake for maintenance should therefore be in relation to the quality of the proteins in the diet. It may be inferred from Brickner's work that high quality proteins quite literally "go further", in nutrition than low quality proteins.

This is an important point to bear in India, since protein rich foods in general and animal protein in particular are quite expensive. A suitable proportion of pulse protein to cereal protein should be found out in order that both proteins are used to the best advantage. It is not possible to suggest at this stage to what extent the quantity of protein intake could be reduced when more than $\frac{1}{3}$ of the total protein is derived from animal protein foods.

Summary and Conclusion

Nitrogen balance experiments were carried out on four adult college women. Subjects were kept first on the basal non-vegetarian diet which supplied one third or more of the total protein intake as animal protein (mutton and cereals), and later on an iso-protein experimental vegetarian diet in which less than 2% of the total protein was from milk (animal protein).

All the subjects excreted less nitrogen in urine per 24 hrs. on the experimental vegetarian diet than on the basal diet but the difference is not significant.

From the observations given in the text and from the discussion it is concluded that the nutritive value of the total protein in a diet (whether vegetarian or non-vegetarian should be determined in terms of essential amino acids composition of the foodstuffs included in the diet as even in the vegetarian diet there will be mutual supplementation in regard to the essential amino acid supply (eg. pulses and cereals).

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Coulometric Estimation of Chromium and Molybdenum

Separately and of Molybdenum in mixtures of the two

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ABSTRACT

Using a Mercury Cathode and Silver-silver Chloride anode, methods have been developed for the coulometric estimation of Chromium and of Molybdenum when present singly and of Molybdenum in presence of Chromium. Conditions for obtaining a good degree of accuracy have been worked out. It is found that Chromium is best estimated in hydrochloric acid-sodium acetate buffer at a pH of about 4 and a Cathode potential of —0·3 V.VS. SCE. For molybdenum when it is present alone the conditions are 0·3M Hydrochloric acid medium of pH 1·5 and a Cathode potential of —0·40 V.VS. SCE. When Chromium also is present the molybdenum estimation is best done at a pH 1·5 to 2·0 and a Cathode potential at —0·4 V.VS. SCE. Under these conditions it is found that molybdenum undergoes one electron reduction from +6Mo to +5Mo and Chromium for +6 Chromium to +3 Chromium.

Introduction

The technique of estimating ionic substances in solution by measuring the quantity of electricity required to effect their complete reaction at an electrode in an electrolysis cell is termed Coulometric analysis. Of the two distinctly different coulometric techniques, namely coulometric titration with constant current and coulometric analysis at controlled potentials of the working electrode, the former has found wide application with the development of polarography and amperometry. The development of the latter technique is still in its initial stages. In the controlled potential coulometry the substance to be determined reacts at 100% current efficiency at a working electrode whose potential is controlled. The completion of the electrolysis is indicated by the current decreasing practically to zero and the quantity of the substance

reacted is calculated from the reading of a coulometer working in series with the cell.

Selection of Working Cathode

Although platinum has been employed as the working electrode (Cathode) by the originator of this method, Lingane has developed the method further and introduced the use of mercury Cathode as the working electrode. The main advantage of the mercury Cathode for Coulometric analyses lies in the fact that the data necessary for the coulometric estimation such as the optimum Cathode potential, the concentration of the electrolyte and nature of the electrolyte etc., have been worked out by investigators in the polarographic field. (J. J. Lingane, 1945). Besides, the mercury Cathode also functions over a wider cathodic potential range with less interference from hydrogen ion reduction, than the platinum cathode.

In addition to its analytical use, mercury cathode coulometry constitutes a valuable technique for establishing conclusively the reduction states that correspond to the polarographic waves observed with dropping mercury electrode especially in those cases where deductions from the Ilkovic equation are indecisive. The reduction states of many organic compounds (I. M. Kalthoff and J. J. Lingane, 1952) have thus been deduced. It has also served to establish the reduction states corresponding to the polarographic waves of $+4$ selenium, $+4$ tellurium (J. J. Lingane and L. W. Niedrach, 1949) $+6$, $+5$ tungsten (J. J. Lingane and Small, 1949) $+6$, $+5$ molybdenum (C. E. Carritt, 1947). In the analytical application and in establishing the reduction states the polarographic half wave potential is a reliable guide. In using this technique for analytical applications J. J. Lingane, as a pioneer in this field has determined coulometrically copper, Bismuth and Lead, and separation of Lead from Cadmium (1945) and the successive determination of Nickel and Cobalt (J. J. Lingane, 1955). These points may be sufficient to indicate the potentialities of the method. In its present state of development the precision cannot be said to be too impressive but there is no theoretical impediment to further improvement of accuracy. The technique also bids fair to be valuable in the successive determination of several metals in a mixture.

The application of this technique to the estimation of the components of steels suggested itself as a fruitful field of investigation. The present paper deals with the estimation of chromium and

molybdenum, two important components of many types of steels. The similarities of molybdenum and chromium offer difficulties in their separation when present together. Hence it was thought that a study of the suitability of the Coulometric technique for estimating the two metals individually and when present together would have some significance in alloy analysis. Although C. E. Carritt (1947) using this method has found out the reduction states for molybdenum, the precise conditions for molybdenum estimation have yet to be worked out. Hence a detailed study of these conditions relating to molybdenum was undertaken as a preliminary to the estimation of molybdenum in presence of chromium. Similarly the conditions for the estimation for chromium were also investigated.

Apparatus

The apparatus used followed the specifications laid down by Lingane (1944, 1945, 1953) in his various publications pertaining to this subject. The essential circuitry for controlled potential coulometric analysis consists of D.C. source, voltage adjuster, the working cell with reference electrode, and the potential measuring device, milliammeter and Voltmeter. Manual control of potential was found sufficient (J. J. Lingane, 1953). The hydrogen-Oxygen coulometer is connected in series with the cell.

The cell employed is that of Lingane's design (1945, 1953). It consists of a cylindrical tube of 100 ml. capacity and the area of the mercury pool Cathode is about 30 $cm.^2$ The mercury solution interface is vigorously stirred (J. J. Lingane, 1944) at constant speed and the formation of droplets of mercury in the swirling motion is avoided. When the mercury solution interface is stirred vigorously, the tip of the reference electrode just touches the mercury surface, so that the Ohmic potential drop that is necessarily included in the measured Cathode potential will be negligibly small.

The Ag anode consists of closely wound helix of 10 gauge silver wire with total surface area of about 55 to 60 cm^2. The solution used contains Chloride ion to make possible the electrode reaction

$$Ag + Cl^- \rightarrow AgCl + e.$$

Experimental

The supporting electrolyte solution is placed in the cell (ca. 80 ml.) and air is removed by passing nitrogen from a cylinder

for a few minutes. The Cathode mercury is then introduced by adjusting the position of the reservoir tube of the cell. The stirrer is started and the tip of the saturated Calomel electrode is placed so that it just touches the mercury surface, when the mercury is in motion. The connection to the coulometer is closed now and the applied voltage adjusted until the potential of the mercury cathode is 0·3 to 0·4 V. more negative than the value at which the determination is to be run and the electrolysis is allowed to run until the current becomes negligibly small. This preliminary electrolysis serves to remove traces of reducible impurities from the solution. Ordinarily the current falls well below 0·5 ma. in 10 mts. In doubtful cases the residual current was ascertained after running this preliminary electrolysis well beyond 30 mts. Without disconnecting the circuit the applied E.M.F. is reduced until the Cathode potential decreases to the desired value and the coulometer burette is read. A known volume, usually not more than 10 ml., of the test solution is then pipetted into the cell. which produces a positive shift of the Cathode potential. The applied E.M.F. is then increased until the desired Cathode potential is reached and the electrolysis is carried out by controlling the potential at this desired value. The electrolysis is stopped when the current has reached the constant minimal value. Ordinarily the electrolysis is over within an hour. The coulometer burette is then read and the value is corrected for standard temperature and pressure. The weight W of the substance reacted is then computed from the relation.

$$W = \frac{VM}{0{\cdot}1739 \times 96500 \times n} = \frac{VM}{16781{\cdot}35\,n}$$

*V Volume of H_2 & O_2 at STP.

M Atomic weight of the metal.

e. The harmful effect on inshore fisheries.

(*Wherever necessary the correction for the residual current is applied).

Determination of Chromium

From neutral and unbufferred solutions of potassium chloride as supporting electrolyte, the polarogram of Chromate consists of

four waves at $-0{\cdot}3$; $-1{\cdot}0$; $-1{\cdot}5$; and $-1{\cdot}7$ V. VS. SCE. (I. M. Kolthoff and J. J. Lingane, 1952). Precise data are available for the polarographic reduction of chromate only in sodium hydroxide and in ammonium chloride-ammonia buffers (I. M. Kolthoff and J. J. Lingane, 1952). However, for Chloride-acid media, bufferred and unbufferred, no precise data are available. We have taken chromate as the starting compound, because it is easy to convert the Chromium in an alloy to Chromate. The acid used must be hydrochloric acid for the reason that it provides also the Cl^- necessary for the Ag — AgCl anode. The anode reaction $Ag + Cl^- \rightarrow AgCl + e$, removes Chloride ion in amount equivalent to the quantity of the reaction at the Cathode without producing any soluble and ionisable substance. The silver Chloride forms an adherent coat on the anode. The quantity of Chloride added must be at least equivalent to the quantity required for the Cathode reaction, but it is desirable to use a 50 to 100% excess. A very large excess of Chloride is also undesirable because of the formation of soluble $AgCl_2^-$ ion which introducing an appreciable amount of dissolved silver into the solution might produce a high residual current.

As no polarographic data are available for the reduction of Chromate in an acid medium and in buffered solutions we have conducted a series of experiments at various potentials and at different pH ranges using 0·3 M HCl as the supporting electrolyte and the observations and conclusions are presented below.

As it is already known that the Chromate gets reduced from Cr^{vi} to Cr^{iii} in unbufferred or neutral Chloride medium (KCl) at a potential of $-1{\cdot}0$ V. VS. SCE we fixed our starting potential at this value and tried various potentials more positive than $-1{\cdot}0$ V. For potentials $-1{\cdot}0$; $-0{\cdot}8$; $-0{\cdot}6$; $-0{\cdot}4$; and $-0{\cdot}3$ V. Vs. SCE even though the initial current was high and proportional to the quantity of the Chromate taken, there was appreciable residual current as high as 3·5 m. amp. even after the electrolysis was prolonged beyond 100 mts. After applying the correction for the residual current the results showed a constant negative error with 2-3 mgms. less than the required value, the calculations being based on the assumption that the electrode reaction is $Cr^{vi} \rightarrow Cr^{iii}$. So coulometric determination of Chromium by mercury Cathode method gives a higher % error when the experiments are conducted in the acid-chloride medium in the potential range $-1{\cdot}0$, to $-0{\cdot}3$ V. VS. SCE.

TABLE I

Potential (Cathode).	Mgms. of Cr. taken	Supporting Electrolyte 0·3 M. HCl. I.C. m. amp.	R.C. m. amp.	Mgms. of Cr. found	Error in Mgms.
—1·0	14·82	200	3·6	12·16	—2·66
—0·75	14·43	200	2·0	11·87	—2·56
—0·60	15·92	200	2·5	13·12	—2·80
—0·50	15·92	200	2·4	13·22	—2·70
—0·40	15·92	200	2·5	13·12	—2·80
—0·30	15·60	175	1·5	13·85	—1·75

*(The time taken for each estimation is about 80-100 mts.).

Although the error may be too high to have any practical value, the consistency of the error indicates that the assumption that the reduction is $Cr^{vi} \rightarrow Cr^{iii}$ is correct. The above results also illustrate that good values cannot be obtained in HCl. medium within this range of potentials. The experiments were now repeated in the same medium to which sodium acetate was added as a bufferring agent. By bufferring the acid-chloride solution, it was observed that the initial current itself was low and the residual current was so small that correction therefor was not necessary in many cases. For the same assumption that the reaction proceeds $Cr^{vi} \rightarrow Cr^{iii}$ the results tend towards higher accuracy at higher pH values. At pH4 it is possible to get good results. But when the concentration of Chromium increases beyond 25 mgms. in the test solution the accuracy tends to fall. So it can be concluded that the estimation of Chromium is feasible with good accuracy in bufferred HCl medium when the pH of the solution is adjusted to 4. The optimum potential of — 0·3 V. VS. SCE as deduced from the values of Table I was found to be quite satisfactory. The reaction is $Cr^{vi} \rightarrow Cr^{iii}$. In all cases the solution turns green and finally to light blue which remains stable. It is possible to estimate Chromium with an accuracy ± 0·2 mgms. deviation.

TABLE II

Coulometric Determinations of Chromium at Various pH's.

Supporting Electrolyte 0·3M. HCl + Sodium Acetate pH 4.

Potential Maintained —0·3 V.VS. SCE.

S. No.	Mgms. of Cr. taken.	I.C. m. amp.	RC m. amp.	Mgms. of Cr. found	error in mgms.
1.	15·6	125	1·0	15·39	—0·21
2.	7·8	70	0·6	7·62	—0·18
3.	10·5	100	0·8	10·29	—0·21
4.	19·50	150	0·6	18·40	—1·10
5.	11·70	80	0·5	11·30	—0·40
6.	7·80	65	0·5	7·612	—0·19

TABLE III

Supporting Electrolyte 0·3M. HCl + Sodium Acetate pH 3.

S. No.	Mgms. of Cr. taken.	I.C. m. amp.	R.C. m. amp.	Mgms. of Cr. found.	error in mgms.
1.	11·89	100	0·8	11·36	—0·53
2.	19·50	160	1·0	18·60	—0·90
3.	15·60	125	1·0	15·10	—0·50
4.	7·80	80	0·8	7·21	—0·59

TABLE IV

Supporting Electrolyte 0·3M. HCl + Sodium Acetate pH 2.

S. No.	Mgms. of Cr. taken.	I.C. m. amp.	R.C. m. amp.	Mgms. of Cr. found.	error in mgms.
1.	15·6	120	1·0	14·25	—1·35
2.	7·8	75	1·5	7·0	—0·80
3.	11·70	100	1·5	10·20	—1·50
4.	19·50	160	2·0	17·80	—1·70

From the results obtained in Tables II, III and IV it appears that the coulometric method yields results of good accuracy ($\pm 0{\cdot}2$ mgms.) when the experiments are conducted in HCl — Acetate media at pH 4, keeping the mercury Cathode at a potential $-0{\cdot}3 \pm 0{\cdot}025$ V. VS. SCE.

Estimation of Molybdenum

C. E. Carritt (1947) investigated the polarography of $+6$ molybdenum and $+3$ molybdenum in sulphuric and hydrochloric acid media and employed the coulometric analysis technique at controlled potential to determine the reduction states. In 0·3 M. HCl the polarogram of $+6$ molybdenum comprises two waves which result from reduction to $+5$ Molybdenum and $+3$ molybdenum with half wave potentials $-0{\cdot}26$ V. and $-0{\cdot}63$ V. VS. SCE. The first diffusion current plateau is not a well defined one but the final diffusion current plateau is quite well defined. With these available polarographic data a coulometric method for the estimation of Molybdenum has been developed in buffered hydrochloric acid medium as described later. As the ultimate aim is to estimate molybdenum and chromium in presence of each other, the feasiblity of this method in the Acetate buffer of various pH's was also studied as chromium gives best results only in the bufferred solution. It is found that molybdenum yields good results in the acid medium and when the pH increases by the addition of Sodium Acetate high positive error was obtained. The potential required in the HCl medium was $-0{\cdot}3$ V. VS. SCE. But the reduction potential becomes more negative as the pH of the media is increased. Thus for HCl medium when bufferred with Acetate to a pH 4 the reduction potential decreases to $-0{\cdot}6$ V. VS. SCE. for the one electron reduction of $Mo^{vi} \rightarrow Mo^{v}$. The observation of C. E. Carritt that in Mo^{vi} changes to Mo^{iii} at a potential of $-0{\cdot}63$ V. VS. SCE. in HCl medium (0·37) giving a green solution apparently does not happen when the solution is bufferred as the reduction in this case is only from $Mo^{vi} \rightarrow Mo^{v}$ giving a yellow solution. For the pure acid medium the reduction potential is $-0{\cdot}3$ V. VS. SCE. which is only $-0{\cdot}04$ V. more than the polarographic half wave potential for the reduction of $+6$ molybdenum to $+5$ molybdenum. When the pH is adjusted by the addition of sodium acetate to 1·5 the potential is $-0{\cdot}40$ V. VS. SCE. Even though it is possible to get good results in the pure acid medium itself for molybdenum we preferred to conduct the experiments for molybdenum at a controlled pH of 1·5 at

a potential of —0·40 V. VS. SCE. because of the possible utility of the method for the estimation of molybdenum and chromium.

TABLE V

Estimation of Molybdenum

Supporting Electrolyte 0·3M. HCl pH 1·5

Potential Maintained —0·40V. VS. SCE.

S. No.	Mgms. of Mo taken	I.C. m. amp.	R.C. m. amp.	Mgms. Mo found.	error in mgms.
1.	53·54	135	0·5	54·16	+0·62
2.	17·97	50	0·5	17·36	—0·61
3.	22·76	60	0·5	22·25	—0·51
4.	46·76	120	0·5	46·26	—0·50
5.	23·66	40	0·8	22·99	—0·67

Under the conditions followed, the results as tabulated above in table V must be considered satisfactory. But when the concentration of Molybdenum increases beyond 50 mgms./100 ml. of the test solution, reproducible results could not be obtained. In the unbuffered acid medium at a potential of — 0·3 V. VS. SCE. the reaction corresponds to one electron reduction and it is possible to obtain results with a deviation of ± 0·6 mgms.

There is always a high residual current in the case of molybdenum at higher pH. When the pH increases the potential at which the reduction takes place also increases, thus we have found that at a pH 4 the potential becomes —0·6 V. VS. SCE. and at higher pH range the initial current was not high enough for the accurate working of H_2 - O_2 coulometer. Hence it is concluded that the estimation of molybdenum at higher pH's is not possible as illustrated in table VI.

TABLE VI

Estimation of Molybdenum in Bufferred Acid Chloride

Supporting Electrolyte 0·3 HCl + Sodium Acetate pH 4.

Potential Maintained —0·6V. VS. SCE.

S. No.	Mgms. of Mo. taken.	I.C. m. amp.	R.C. m. amp.	Mgms. of Mo. found	Error in mgms.
1.	44·7	50	3·5	43·50	+1·2
2.	54·5	35	2·2	52·01	+2·5

It can also be seen that the final residual current was too high. The initial residual current of the supporting electrolyte also was high.

In Mixtures:

From the above considerations it is established that the reduction potentials in the medium chosen for hexavalent chromium and hexavalent molybdenum are too close to permit their separate estimation when present together. To eliminate this difficulty hexavalent Chromium is reduced by chemical means to trivalent Chromium without simultaneously reducing hexavalent molybdenum. This is achieved by taking Cr^{vi} and Mo^{vi} solutions to which appropriate amount of concentrated HCl. is added and a few drops of alcohol and heated over a water bath for 50-60 minutes when all the $+6$ Chromium gets reduced to $+3$ Chromium. This solution is made up and used as the stock solution whose activity is accurately assessed. The final acidity of the stock solution prepared is 1·5 N. The ratio of molybdenum to Chromium is as high as 6 : 1 which may roughly correspond to the ratio obtained in some steels.

In estimating molybdenum in presence of Chromium after reducing $Cr^{vi} \rightarrow Cr^{iii}$ by chemical means, at a potential of $-0{\cdot}3$V. VS. SCE. in purely acid medium we found there was high positive error with high residual current, whereas for molybdenum alone it was possible to get accurate results in the medium. Even though the initial current was satisfactorily high and varied proportional to the concentration, the final residual current was too high. After applying correction for this residual current and calculations made on the basis of one electron reduction of molybdenum ($Mo^{vi} \rightarrow Mo^{v}$) it was found that there was always a high positive error from which it was concluded that it was not possible to estimate molybdenum in presence of trivalent Chromium accurately in pure acid-Chloride medium, even though the medium proved quite good at that potential when molybdenum alone ($Mo^{vi} \rightarrow Mo^{v}$) was present. The following table substantiates the above statement.

A complete investigation of the conditions of estimation of molybdenum in presence of chromium revealed that whereas molybdenum when present alone could be accurately estimated at low pH (> 1), the presence of Chromium is found to shift the pH range for accurate estimation to the value 1·5-2 (Table VIII). In other pH ranges errors beyond permissible limits were obtained.

TABLE VII

Estimation of Molybdenum in Presence of Chromium

Supporting Electrolyte 0·3M. HCl.

Potential Maintained —0·3V. VS. SCE.

S. No.	Mo taken mgms.	Cr. Present mgms.	I.C. m. amp.	R.C. m. amp.	Mo. found mgms.	Error mgms.
1.	15·56	14·19	35	1·5	17·15	+1·59
2.	17·24	14·82	35	1·5	19·21	+1·97
3.	17·01	14·70	35	2·0	18·85	+1·84
4.	33·12	14·20	45	1·5	38·11	+4·99

TABLE VIII

Estimation of Molybdenum in Presence of Chromium at Various pH's.
Supporting Electrolyte 0·3M. HCl + Sodium acetate
Potential Maintained —0·6V. VS. SCE.

S. No.	pH	Mo. taken mgms.	Cr. present mgms.	I.C. m. amp.	R.C. m. amp.	Mo. found mgms.	error mgms.
1.	4·0	21·36	3·82	30	3·10	24·37	3·01
2.	2·0	21·36	3·82	40	2·0	21·60	0·24
3.	1·5	19·62	3·50	40	1·9	19·43	—0·19
4.	1·5	18·31	3·20	40	3·0	18·14	—0·17
5.	1·0	28·34	5·07	60	3·0	31·29	2·95

The optimum potential at which molybdenum could be estimated accurately in presence of Chromium was found to be —0·4V. VS. SCE. A more — ve potentials upto — 0·6 the error was found to be high. More positive potentials were ruled out because of the excessive slowness of the reaction.

TABLE IX

Estimation of Molybdenum in Presence of Chromium under Optimum Conditions

Supporting Electrolyte 0·3M. HCl + Sodium acetate, pH. 1·5
Potential Maintained —0·4V. VS. SCE.

S. No.	Mo. taken mgms.	Cr. present mgms.	I.C. m. amp.	R.C. m. amp.	Mo. found mgms.	Error mgms.
1.	17·44	3·12	30	1·70	17·1	—0·34
2.	26·22	5·85	40	2·00	26·46	+0·26
3.	37·27	7·32	50	2·40	37·03	—0·24
4.	28·34	5·07	35	2·40	28·42	+0·08

From the results obtained in Table IX it will be clear that the coulometric method yields results of good accuracy for the estimation of molybdenum in presence of Chromium, when the experiments were conducted in HCl- Acetate medium at a pH of 1·5 to 2·0 keeping the mercury Cathode at a constant potential —0·4V. VS. SCE.

While the conditions for the coulometric estimation of molybdenum and of Chromium separately and of molybdenum in presence of Chromium have thus been completely worked out, a method for the estimation of chromium in presence of molybdenum remains to be evolved.

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Petrochemistry of the Granites and Associated Rocks of Chamundi Hill, Mysore State

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ABSTRACT

The chemical analyses of all the granites, gneisses and other rock types of the Chamundi area are brought together for the purpose of comparison and discussion of origins. Variation diagrams, linear and trilinear, after various authors, Harker, Brammall, Holmes, Niggli, Reynolds, Barth and Lapadu-Hargues have been prepared and studied. The gneisses and the granites do not show inter-relationship, but the hornblende schists and amphibolites have been migmatised to form gneisses. The granites themselves constitute a differentiation series with metasomatic effects induced by later intrusion of aplites and pegmatites. There is no convincing evidence that the Chamundi granite is a product of granitisation of the country rocks.

To investigate the genesis of the granites of Chamundi, representatives of each group were chosen for chemical analysis. Information concerning the analytical data of the rocks of Chamundi is lacking in literature. 20 rocks were chemically analysed and they are presented in Table I.

Analytical Data

The range of variation in granites of the several oxides is as follows :—

SiO_2	65·77 — 73·69%	Fine-grained Granodiorite-pink granite.
FeO, Fe_2O_3	4·45 — 1·16%	Pinkish grey granite-aplite.
MgO	3·03 — 0·36%	Granodiorite-coarse pink granite.
CaO	3·07 — 1·32%	Pink porphyritic granite-aplite.
Na_2O	5·61 — 4·12%	Aplite-grey porphyritic granite.
K_2O	2·72 — 4·00%	Pinkish grey granite-grey porphyritic granite.
Al_2O_3	15·51 — 14·44%	Grey porphyritic granite- aplite.

TABLE I

	B/11	B/88	B/137	B/24	B/27	B/71	B/65	B/18	B/26	B/33	B/135	B/66	B/134	B/112	B/74	B/162	B/108	B/70	B/32
SiO_2	76·69	73·59	72·59	70·41	70·21	70·15	68·84	68·34	68·16	68·16	68·47	67·06	65·77	66·83	65·70	60·06	56·46	44·32	41·64
Al_2O_3	15·06	14·58	14·44	14·44	14·26	17·56	14·67	16·59	14·92	14·88	14·72	15·11	15·07	13·98	14·08	15·89	14·35	15·01	15·49
Fe_2O_3	0·42	0·67	0·83	1·58	1·80	0·09	0·94	1·44	2·98	1·31	1·54	1·96	1·26	0·57	2·28	2·12	4·12	1·27	4·01
FeO	0·43	0·50	0·33	1·22	1·22	0·61	1·54	1·14	0·16	1·26	1·06	2·49	1·33	2·79	4·73	4·97	9·39	12·52	12·06
MnO	0·31	0·01	0·03	0·03	0·04	Nil	0·06	0·04	0·06	0·21	0·33	0·09	0·05	0·07	0·09	0·37	0·78	0·16	0·11
MgO	0·74	0·36	0·52	1·04	1·16	2·48	1·59	1·17	1·24	1·78	1·15	1·75	3·03	3·56	4·63	4·63	8·37	8·60	9·60
CaO	1·21	1·91	1·32	3·18	3·31	3·93	2·92	2·25	3·22	3·28	3·03	2·87	2·78	4·90	3·40	5·25	3·18	13·00	4·31
Na_2O	4·22	4·44	5·61	3·80	3·87	4·35	4·43	4·10	4·26	4·89	4·76	4·51	5·51	3·65	1·39	4·53	2·01	2·14	1·52
K_2O	3·22	3·69	3·76	4·03	3·39	0·46	3·65	4·19	4·51	3·75	4·26	2·72	3·50	2·11	1·49	1·04	0·78	0·89	7·49
TiO_2	0·05	0·09	0·03	0·18	0·29	Nil	0·19	0·19	0·27	0·25	0·22	0·42	0·39	0·48	0·78	0·62	0·80	1·30	1·06
P_2O_5	Trace	0·03	Trace	0·17	0·40	Nil	0·09	0·09	Trace	N.D.	0·04	0·04	0·13	0·13	0·18	0·13	N.D.	0·18	Trace
H_2O^+	0·35	0·19	0·41	0·33	0·39	0·37	0·83	0·34	0·62	0·57	0·33	0·77	0·60	0·71	1·44	0·86	0·10	0·73	1·95
H_2O^-	0·12	0·25	0·51	0·11	0·09	0·16	0·13	0·13	0·05	—	0·10	0·38	0·08	0·12	0·11	0·02	0·15	0·16	0·29
Total	99·82	100·31	100·38	100·52	100·43	100·16	99·88	100·01	100·45	100·34	100·01	100·17	99·50	99·90	100·30	100·49	100·49	100·28	99·53

B/11 — Fine-grained pink granite
B/88 — Coarse-grained pink granite
B/137 — Aplite
B/24 — Grey porphyritic granite
B/27 — Granodiorite
B/71 — Pegmatite

B/65 — Porphyritic pink granite
B/18 — Porphyritic grey granite
B/26 — Porphyritic grey granite
B/33 — Coarse-grained grey granite
B/135 — Granodiorite
B/66 — Pinkish grey granite
B/134 — Fine-grained grey granite

Analyst — S. K. BABU

B/112 — Hornblende Gneiss
B/74 — Hornblende Gneiss
B/162 — Hornblende Biotite Gneiss
B/108 — Garnetiferous Biotite Gneiss
B/70 — Amphibolite
B/32 — Basic Biotite Patch from Granite

Analyst — C. E. NEHRU

From the above data we find that the rocks of Chamundi vary in chemical composition between silica rich alkali granites and leucogranites chemically comparable to adamellites and granodiorites.

All the granites show markedly alkaline features reflected in their chemistry and mineralogy. Alkali felspars predominate over calc-alkali types. Albite occurs throughout and during the intrusion period tends to increase at the expense of potash felspars.

Variation

To study the possibility of differentiation in the granites of Chamundi, the weight percentages of all the oxides are plotted against SiO_2 in the variation diagram Fig. 1. The points are

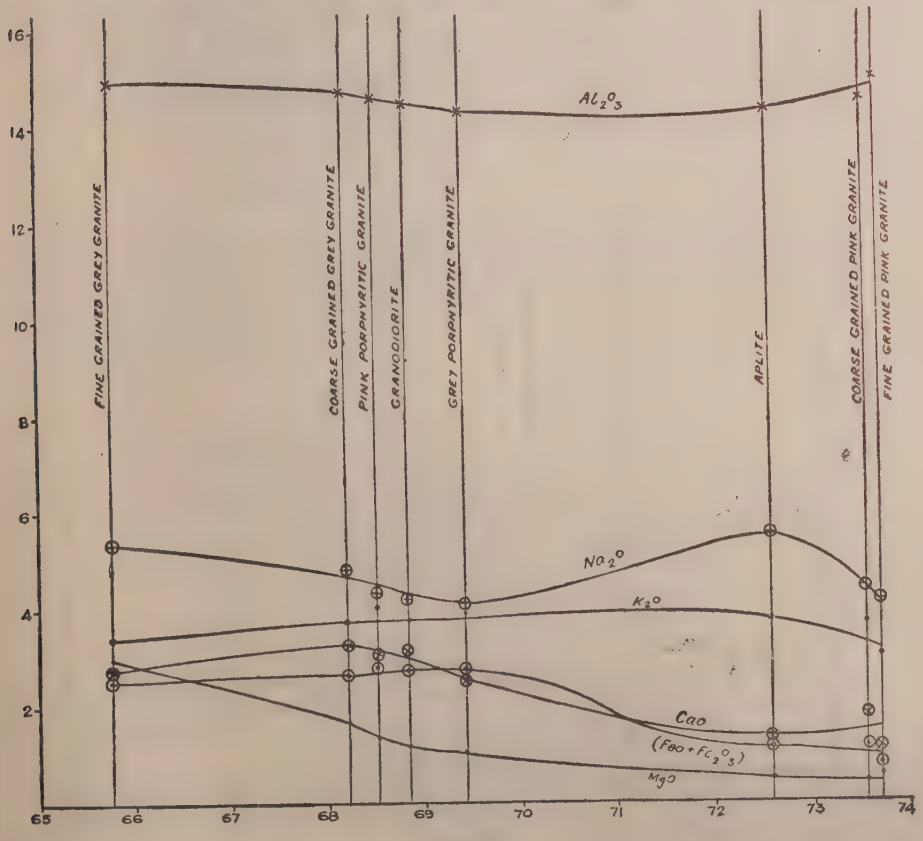

Fig 1.

scattered and the curves are generally smooth, a feature characteristic of differentiation (Ghosh 1934). The nature and shape of the curves resemble very closely, the curves drawn for Carnmenallis granites by Ghosh (1934), but for some minor differences.

The general trend of variation is as SiO_2 increases, the percentages of FeO, Fe_2O_3, MgO, CaO and TiO_2 decrease. There is a reciprocal variation between soda and potash. According to Ghosh (1934) these are features of differentiation. No xenoliths in these rocks have been found which could have modified the initial character of the alkalies.

Arthur Holmes (1921) has observed that the most significant feature of the two suites of rocks namely alkaline and calc-alkaline is the SiO_2 percentage at the intersection of lime curve with those of alkalies. He has fixed 54% SiO_2 for alkaline and 66% SiO_2 for calc-alkaline suite. In the above variation diagram, we notice that the lime curve does not intersect the alkali curves and also the alkali curves lie above the lime curve.

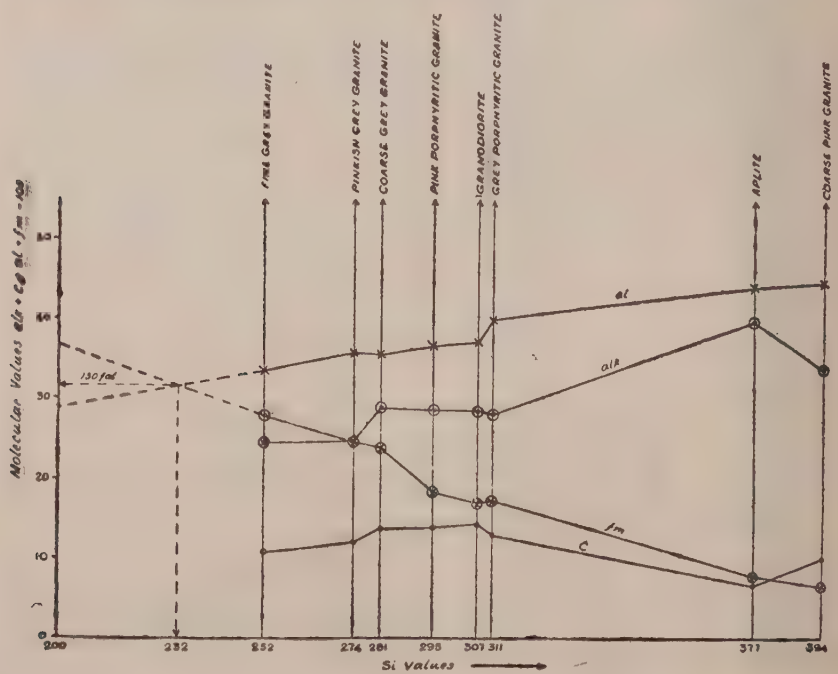

FIG. 2

The Niggli molecular values calculated for these rocks agree with those of common magma types of pacific suite (viz. trandhjemetic, granodioritic, leucogranitic and granitic).

The Niggli values for the granites are plotted in the variation diagram Fig. 2. The curves resemble those of Lassen peak as given by Burri and Niggli (1945).

The intersection of al with fm curve (isofalic point) is at the si value 232. The values fm: al is 32 and corresponds with that of Pacific suite. Burri and Niggli (1945) give the following isofalic and corresponding si values for various provinces.

	al : fm	si
Lassen Peak (Pacific) ..	32	176
Highwood Mountains (Mediterranean) ..	29	148
Tristandacunha (Atlantic) ..	30	135

The k-mg values are plotted in the corresponding diagram of Lassen Peak Fig. 3. In the k-mg diagram the granites of Chamundi cluster near the field of concentration of points for Lassen Peak.

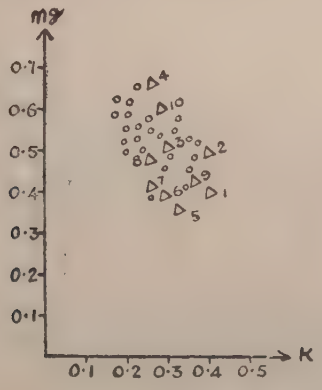

Fig 3

In order to know whether the granites of Chamundi belong to the eruptive rocks, the al, alk and c/fm values were plotted in the Niggli diagram (given by Johannsen). It was found that all the granites lie in the eruptive field excepting B/71 and B/24 Fig. 4.

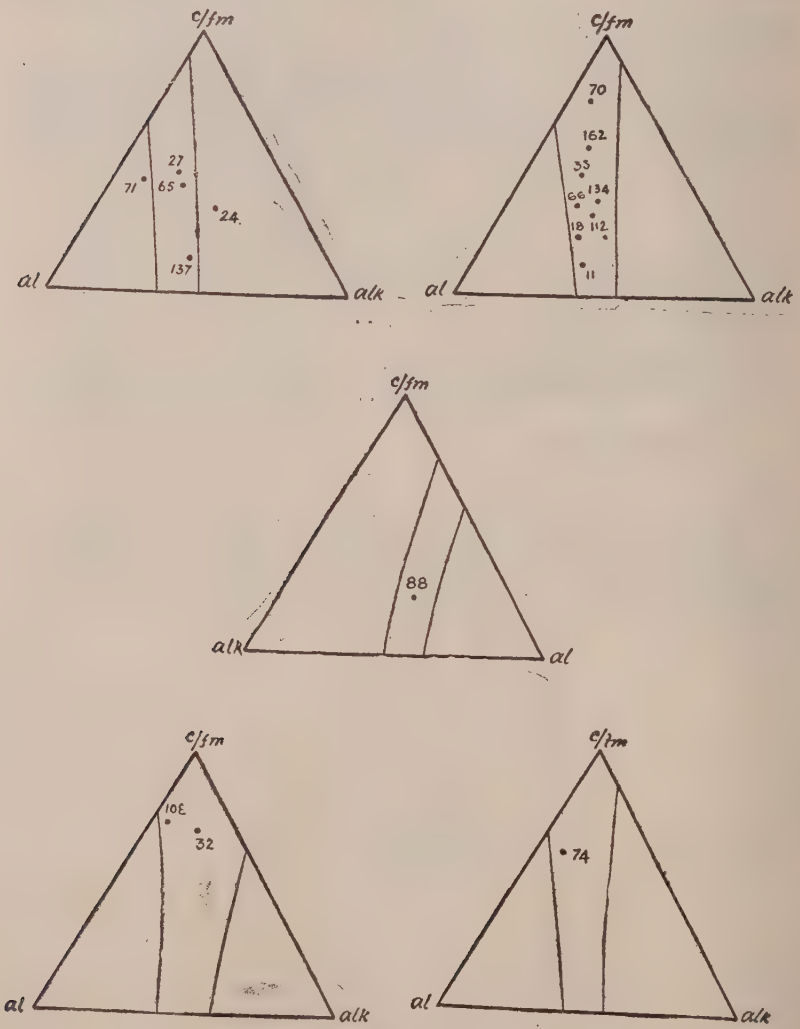

Fig. 4

When the Or-Cor, Ab, An-Fem values are plotted in the Brammall diagram (1932) Fig. 5 it is noticed that there are two

distinct fields namely all the granites occupy the igneous field. The basic schlieren occupy the sedimentary field assigned for shales in Brammall diagram of Dartmoor granites (1932).

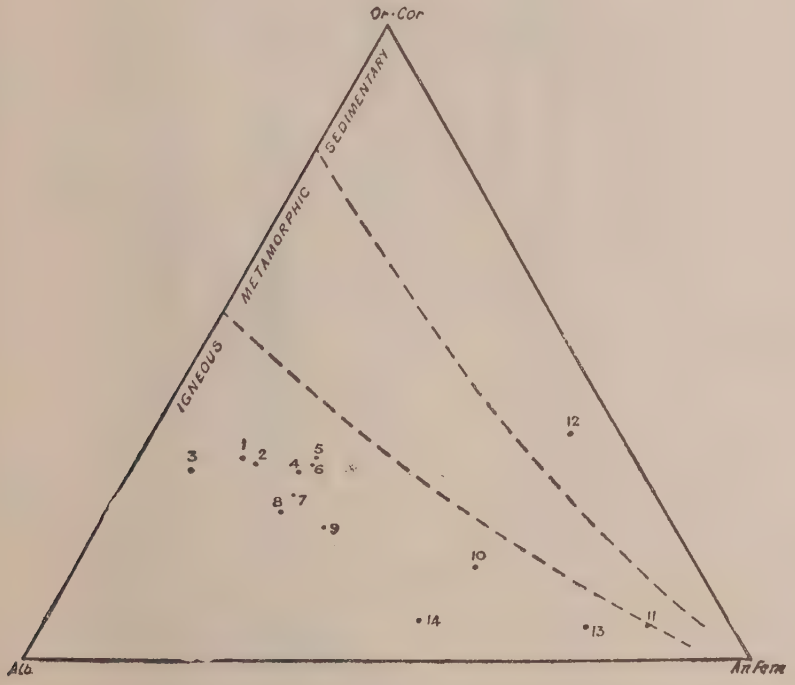

Fig. 5

1. Fine grained pink granite; 2. Coarse grained pink granite; 3. Aplite; 4. Granodiorite; 5. Grey porphyritic granite; 6. Pink porphyritic granite; 7. Coarse grained grey granite; 8. Fine grained grey granite; 9. Pinkish grey granite; 10. Gneiss; 11. Amphibolite; 12. Biotite Schlieren; 13. Garnetiferous Biotite Gneiss; 14. Biotite gneiss.

Recently Lapadu-Hargues (1945) has investigated the composition of a great number of silica-alumina rocks, from unaltered sedimentary rocks to mica schists, also gneisses and granites and has plotted the percentages of alkalies against alumina Fig. 6 a & b. He found that the areas over which the points were distributed became smaller as the degree of metamorphism increased; in other

words the dispersion of analyses of rocks of the same type from different countries of the world decreases as the intensity of

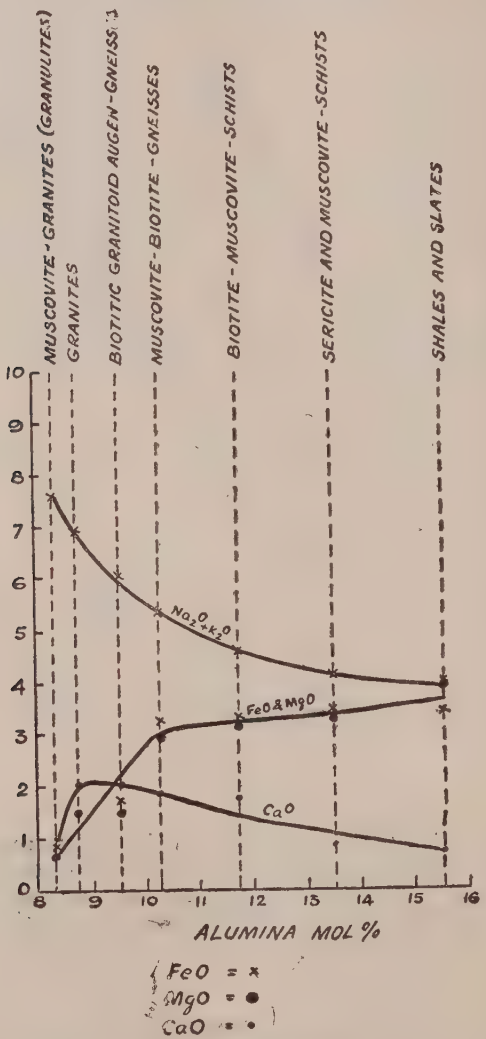

FIG. 6(a)

metamorphism increases. Moreover, he found that no break occurs in the lime at the boundary between granite and gneiss.

To know whether in Chamundi a continuous metamorphic grade persists between the granites and gneisses without a distinct break, the molecular percentages of (FeO and MgO), (CaO), and (Na_2O and K_2O) were plotted against Al_2O_3 Fig. 6b.

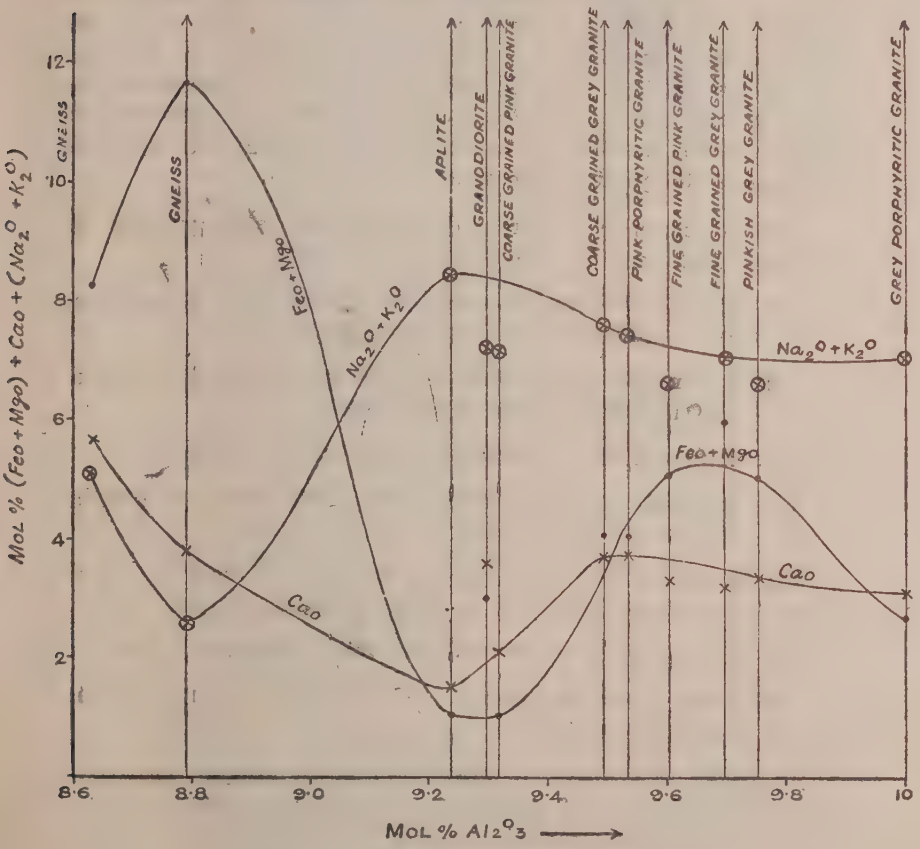

Fig. 6(b)

When they are plotted, we notice that the shapes of the (FeO and MgO), (CaO), (Na_2O and K_2O) curves do not conform to the shapes of curves drawn by him for crystalline schist series comprising shale to granites. The curves from gneiss to granites are not continuous. On the other hand a distinct break exists between the granites and gneisses, thereby, suggesting that granites and gneisses are two separate entities. But among the different granites themselves we find a definite metamorphic grade. The

curves of ($FeO + MgO$), (CaO), (Na_2O and K_2O), very nearly resemble the curves given by Lapadu-Hargues. Lapadu-Hargue's diagram (1945) is chiefly meant to study granitisation from sedimentary shale to muscovite granite through transitional members. In the diagram instituted for granites and gneisses of Chamundi, as there is a distinct break between them, it can be concluded that granites are not formed from gneisses by the process of granitisation. On the other hand, the similarity of the curves among granites with those of Lapadu-Hargues suggests that there may be granitisation to some extent within the different members of the granite themselves.

The evidences in favour of differentiation of the granites of Chamundi Hills may be summarised as follows:—

(1) Qualitatively and quantitatively the mineral composition corresponds to granite and granodiorite.

(2) Myrmekite intergrowths and corroded plagioclase grains are suggestive of late crystallisation.

(3) A few of the microclines exhibit carlsbad twinning.

(4) Oriented perthitic stringers of albite occur in potash felspar which are the result of exsolution.

(5) Small euhedral grains of zircons and apatite occur uniformly distributed in some of the rock types.

(6) Scattering of points in the variation diagram.

The above evidences point to differentiation of some of the rocks of Chamundi.

According to the second school of thought granites are produced by the process of granitization. Reynolds (1946) investigated the geochemical changes leading to granitization. She has collected together many chemical analyses relating to reaction effects of granites on various types of country rocks in order to determine whether there is any systematic sequence of changes when rocks are granitized. According to Reynolds granitisation takes place in two distinct stages. During the first stage the rocks become molecularly desilicated relative to bases present sometimes to such an extent as to become chemically under-saturated. During the second stage the altered rocks get granitized and SiO_2 and one of the alkalies are added, whilst alumina, femic constituents and minor constituents TiO_2, P_2O_5, MnO decrease. Such a decrease

of any constituent below the amount present in the parent rock is referred to by Reynolds as geochemical depression and increase of any constituent beyond the amount present in the rock as geochemical culmination.

Reynolds is the bravest and foremost among the transformists and she has further given a pictorial view of all these changes by plotting the analyses of individual areas on Von Wolff's diagram. In order to study the possibility whether Chamundi granites originated from the adjoining gneisses by the process of granitisation, the sequence of geochemical changes from amphibolite to gneiss and to granite were studied after Reynolds (1946) and is presented in Table 2 a & b, on pages 544-45.

From amphibolite to hornblende gneiss it is geochemical culmination of silica and depression of cafemic and minor constituents. This means that the amphibolite gets felspathised by acidic alkaline solutions resulting in hornblende gneiss. From hornblende gneiss to garnetiferous gneiss it is a geochemical depression of silica and culmination of cafemic and minor constituents. That is, the hornblende gneiss gets basified whereby hornblende breaks up and its MgO, Al_2O_3, and Fe_2O_3 go to form garnet, while with the help of the invading solutions, and FeO and Al_2O_3 of hornblende, biotite develops. From garnetiferous gneiss to biotite gneiss it is a geochemical culmination of silica and depression of cafemic and minor constituents. The sequence of geochemical changes from amphibolite to biotite gneiss indicates that the amphibolite is converted in successive stages resulting ultimately in biotite gneisses. But when a similar study of geochemical sequence is instituted between biotite gneiss and non-porphyritic grey granite and pink granite (average) we find the sequence do not satisfy the conditions of granitisation. Even within the granites that is between non-porphyritic and porphyritic the conditions do not satisfy granitisation.

To study whether granites have resulted from gneisses, additive and subtractive diagrams Fig. 7 between amphibolite and gneiss and between gneiss and granites were perpared in order to see whether a rock corresponding very similar in composition to a pegmatite could be obtained. When an additive diagram between amphibolites and gneiss was prepared, a rock very closely similar in composition to a pegmatite chemically analysed was obtained thereby proving that gneisses have resulted from amphibolites by the process of injection and pegmatisation.

TABLE 2(a)

	Amphibolite.	Hornblende gneiss.	Garnet-biotite gneiss.	Biotite gneiss.	Average non-porphyritic grey granite.	Average pink non-porphyritic granite.	Average grey porphyritic granite.	Average pink porphyritic granite.	Average pink and grey non-porphyritic granite.	Average pink and grey porphyritic granite.
SiO_2 ..	44·32	66·83	56·46	60·06	68·45	73·64	69·37	68·50	71·04	68·93
TiO_2 ..	1·30	0·39	0·80	0·62	0·25	0·07	0·18	0·23	0·17	0·20
Al_2O_3 ..	15·01	13·98	14·35	15·89	14·68	14·82	15·51	14·79	14·75	15·15
Fe_2O_3 ..	1·27	0·57	4·12	2·12	1·43	0·54	1·51	1·96	0·98	1·73
FeO ..	12·52	2·79	9·39	4·97	1·19	0·46	1·18	0·85	0·82	1·01
MnO ..	0·16	0·07	0·78	0·37	0·19	0·16	0·04	0·06	0·16	0·05
MgO ..	8·60	3·57	8·37	4·63	1·46	0·55	1·10	1·41	1·00	1·25
CaO ..	13·00	4·90	3·18	5·25	3·22	1·56	2·71	3·07	2·39	2·89
Na_2O ..	2·14	3·65	2·01	4·53	4·60	4·33	4·21	4·35	4·51	4·23
K_2O ..	0·89	2·11	0·78	1·04	3·78	3·45	4·11	4·08	3·61	4·09
P_2O_5 ..	0·18	0·13	N.D.	0·13	0·20	0·03	0·13	0·09	0·11	0·11

TABLE 2 (b)

	SiO_2	Al_2O_3	Fe_2O_3	FeO	MgO	CaO	Na_2O	K_2O	TiO_2	MnO	P_2O_5
Amphibolite Hornblende Gneiss.	⊕	−	−	⊖	⊖	⊖	+	⊕	⊖	−	−
Hornblende Gneiss. Garnetiferous biotite Gneiss.	⊖	+	⊕	⊕	⊕	−	−	⊖	+	⊕	+
Garnetiferous biotite Gneiss. Biotite Gneiss.	⊕	+	⊖	⊖	⊖	⊖	⊕	+	−	⊖	
Biotite Gneiss. Average of non-porphyritic grey granites.	⊕	−	−	⊖	⊖	⊖	+	⊕	−	⊖	+
Non-porphyritic grey Non-porphyritic pink granites (average)	⊕	−	−	+	+	⊖	−	−	−	⊖	⊖
Non-porphyritic grey granites Porphyritic grey Granites	+	+	+	−	−	−	−	−	⊖	−	−
Non-porphyritic pink granite Porphyritic pink granites	⊖	−	⊕	+	+	⊕	+	+	⊕	−	+
Non-porphyritic pink and grey granite porphyritic pink and grey granite.	⊖	+	+	+	+	+	−	+	+	⊖	⊖

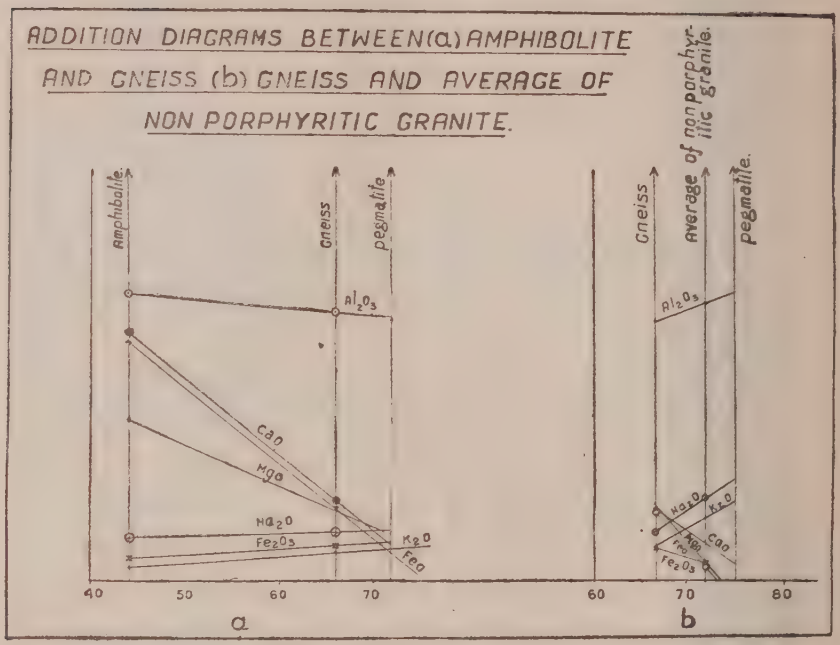

FIG. 7

	Amphibolite.	Gneiss (average).	Pegmatite.	Analysis obtained from additive diagram.
SiO_2	.. 44·32	66·26	70·15	72·00
Al_2O_3	.. 15·01	14·03	17·56	13·80
Fe_2O_3	.. 1·27	1·92	0·09	2·00
FeO	.. 12·52	3·76	0·61	1·50
MgO	.. 8·60	4·09	2·48	2·50
CaO	.. 13·00	4·15	3·93	4·01
Na_2O	.. 2·14	2·52	4·35	3·00
K_2O	.. 0·89	1·80	0·46	1·50

Q.L.M. values after Von Wolff were calculated for the amphibolite, hornblende gneiss, pegmatite. These values are plotted in the Von Wolff Q.L.M. diagram after the method of Reynolds (1946) Fig. 8. It is found that the points take up positions, suggesting, that the trend of geochemical changes is to convert amphibolites into hornblende gneisses by successive stages.

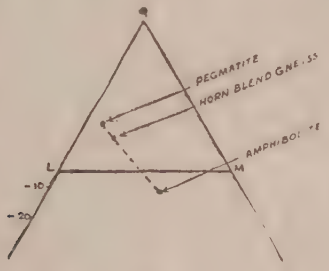

Fig. 8

When a similar additive diagram is prepared between gneisses (average) and granites porphyritic and non-porphyritic (average) we find that a rock similar in composition to SiO_2 prcentage of pegmatite is obtained. This suggests that some of the granites if not all have resulted by the metasomatic granitisation of the gneisses.

In petrographic calculations it is often important to compare rocks of equal volume because most replacement in rocks and mineral deposits take place without appreciable change often with preservation of most delicate structures. A quantitative idea of the extent of diffusion affect has been rendered possible by Barth's (1951) standard cell method.

In order to study the metasomatic changes undergone among the granites and gneisses of Chamundi the standard cell was calculated for all the different types of granites, gneisses and amphibolites and is presented in Table 3 (a & b, on pages 548-51).

From the table we notice that the valencies of the added and subtracted materials in the granites are also equal to one another excepting for minor differences of one or two which is a common feature while dealing with any rock series. This clearly suggests that the granites have undergone volume changes after their differentiation. The presence of sericitisation and development of muscovite, abundance of epidote and turbidity of the plagioclase

TABLE III (a)

STANDARD CELL FOR VARIOUS GRANITES AND GNEISSES ON 160 CATION BASIS

1. Amphibolite ..	$K_{1.20}Na_{3.98}$	$Ca_{13.67}Mg_{12.62}$	$Fe'''_{12.26}Al_{17.24}$	$Ti_{1.19}Si_{43.33}((OH)_{10.32}$	$O_{149.68})_{160}$
2. Hornblende gneiss ..	$K_{1.64}Na_{2.36}$	$Ca_{5.13}Mg_{5.95}$	$Fe'''_{5.13}Al_{14.17}$	$Ti_{0.51}Si_{55.97}((OH)_{17.72}$	$O_{142.28})_{160}$
3. Biotite gneiss ..	$K_{1.18}Na_{7.82}$	$Ca_{5.03}Mg_{6.15}$	$Fe'''_{5.85}Al_{16.70}$	$Ti_{0.42}Si_{53.58}((OH)_{10.28}$	$O_{149.72})_{160}$
4. Hornblende-biotite gneiss ..	$K_{1.17}Na_{3.15}$	$Ca_{4.70}Mg_{4.75}$	$Fe'''_{2.79}Al_{14.64}$	$Ti_{0.32}Si_{59.48}((OH)_{8.32}$	$O_{151.68})_{160}$
5. Grey Porphyritic granite ..	$K_{4.80}Na_{7.21}$	$Ca_{2.19}Mg_{1.55}$	$Fe'''_{1.85}Al_{17.41}$	$Ti_{0.16}Si_{60.81}((OH)_{5.56}$	$O_{154.44})_{160}$
6. Coarse grained grey granite ..	$K_{4.19}Na_{8.49}$	$Ca_{3.17}Mg_{2.36}$	$Fe'''_{1.94}Al_{15.69}$	$Ti_{0.16}Si_{61.02}((OH)_{6.66}$	$O_{153.34})_{160}$
7. Fine grained grey granite ..	$K_{4.03}Na_{9.68}$	$Ca_{2.72}Mg_{4.09}$	$Fe'''_{1.94}Al_{16.11}$	$Ti_{0.27}Si_{59.67}((OH)_{7.18}$	$O_{152.82})_{160}$
8. Pink porphyritic granite ..	$K_{5.15}Na_{7.40}$	$Ca_{3.06}Mg_{1.66}$	$Fe'''_{2.22}Al_{15.66}$	$Ti_{0.21}Si_{60.92}((OH)_{7.76}$	$O_{152.24})_{160}$
9. Coarse grained pink granite ..	$K_{4.08}Na_{7.43}$	$Ca_{1.78}Mg_{0.47}$	$Fe'''_{0.94}Al_{14.97}$	$Ti_{0.04}Si_{64.24}((OH)_{5.05}$	$O_{154.98})_{160}$
10. Fine grained pink granite ..	$K_{3.56}Na_{7.12}$	$Ca_{1.10}Mg_{0.94}$	$Fe'''_{0.65}Al_{15.39}$	$Ti_{0.05}Si_{64.31}((OH)_{5.44}$	$O_{154.66})_{160}$
11. Medium grained pinkish grey granite ..	$K_{3.14}Na_{7.90}$	$Ca_{2.82}Mg_{2.33}$	$Fe'''_{3.15}Al_{16.01}$	$Ti_{0.27}Si_{60.37}((OH)_{4.52}$	$O_{155.48})_{160}$

TABLE III (b)

	Amphibolite hornblende gneiss.		Hornblende gneiss biotite gneiss.		Hornblende biotite gneiss biotite gneiss.		Hornblende biotite gneiss porphyritic granite.	
	+	−	+	−	+	−	+	−
Si	12·64	—	—	2·39	—	5·90	1·33	—
Al	—	3·07	2·53	—	0·10	—	2·77	—
Ti	—	0·68	—	0·09	2·06	—	—	0·16
Fe'''	—	7·13	0·75	—	3·06	—	—	0·94
Mg	—	6·67	0·20	—	1·40	—	—	3·20
Ca	—	8·54	—	0·10	0·33	—	—	2·51
Na	—	1·62	5·46	—	4·67	—	4·06	—
K	0·44	—	—	0·46	0·01	—	3·63	—
OH	7·40	—	—	7·44	1·96	—	—	2·76
Total	65·80	64·35	15·70	25·46	26·82	23·60	21·92	20·40

TABLE III (b) contd.

	Grey porphyritic granite coarse grey granite.		Coarse grey granite fine grey granite.		Pink porphyritic granite coarse pink granite.		Coarse pink granite fine pink granite.		Fine pink granite pinkish grey granite.	
	+	−	+	−	+	−	+	−	+	−
Si	0·21	—	—	1·35	3·32	—	0·07	—	—	3·94
Al	—	1·72	0·42	—	—	0·69	0·42	—	0·62	—
Ti	—	—	0·11	—	—	0·17	0·01	—	0·22	—
Fe'''	0·09	—	—	—	—	1·28	—	0·29	2·50	—
Mg	0·17	—	1·73	—	—	1·19	—	0·68	1·72	—
Ca	0·26	—	—	0·45	—	1·28	0·47	—	1·39	—
Na	1·28	—	1·19	—	0·03	—	—	0·31	0·78	—
K	—	0·61	—	0·16	—	1·07	—	0·52	—	0·42
OH	1·11	—	0·52	—	—	2·74	0·42	—	—	0·92
Total	4·36	5·77	6·87	6·46	13·31	15·24	2·94	3·06	17·24	15·16

lend further support for metasomatism of granites. Hence it can be concluded that within the granites, both pink and grey series have undergone metasomatic changes during the intrusion period of the magma, probably represented by the aplitic and pegmatitic phase of the fine-grained granites.

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Development of the Monaxonid Sponge *Lissodendoryx Similis* Thiele[1, 2]

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ABSRACT

Sexually produced larva of *Lissodendoryx similis* THIELE, at the time of liberation is a stereogastrula and possesses a well developed outer layer and an inner mass of cells with a bundle of spicules at the posterior end. It has a total free swimming period of nearly forty hours. Metamorphic changes start appearing even during the motile phase and the nuclei of the nuclear stratum are seen to commence their migration into the rod stratum of the epithelium outwardly and into the mesenchyme inwardly. Metamorphosis is described in four distinct stages. As in most sponges studied, in *Lissodendoryx similis* also the essential feature of metamorphosis seems to be the migration of nuclei and cells, almost bringing about an inversion of layers. Choanocytes are formed by the immigrated epithelial nuclei together with the mesenchymal cytoplasm. The epidermis of the adult is formed by the mesenchymal cells which have migrated to the surface, as in several other sponges. Previous work on the development of sponges is discussed in relation to the present study.

Introduction

Larval development in Monaxonid sponges has been studied by many. (Delage, 1890, 1891, 1892, 1893, 1899, Maas, 1890, 1891, 1892, 1893, 1895, Evans, 1899, Ganin, 1879, Goette, 1886; Wilson, 1891, 1894, 1935, Sivaramakrishnan 1951).

A characteristic feature in the larval development of most of the sponges studied is the reversal of larval tissue so that the cells in the interior of the larva migrate towards the outer side to form the adult epidermis and cells or nuclei of the cells of the outer layer migrate inside to give rise to the choanocytes. This feature appears to be common in all monaxonid sponges with the

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exception of *Spongilla fluviatalis* (in which Ganin (1879) and Goette (1886) found that the outer cells of the larval tissue remain unchanged in position and give rise to the epidermis. However, as, many variations have been noted by the different authors in the process of the inversion of layers it was thought that the larval development in a sponge-like *Lissodendoryx similis,* which occurs in Madras would be of interest.

Considerable difference of opinion prevails regarding the origin of choanocytes during the larval development of sponges. As discussed later, there are three different accounts (Delage, 1890, 1891, 1892, 1893· 1899; Maas, 1890, 1891, 1892, 1893; 1895; Evans; 1899, Ganin, 1879, Goette, 1886, Wilson, 1891, 1894, 1935; Sivaramakrishnan, 1951), based on the larval development of different species (*Esperella sordida, Spongilla fluviatalis, Esperia lorenzi, Spongilla lacustris, Esperella fibrexilis, Tedania brucei, Mycale syrinx,* and *Callyspongia diffusa*). Since the development of only one Madras form (*Callyspongia diffusa*) has been studied so far (Sivaramakrishnan, 1951) and since only larvae from gemmules have been studied it was considered worthwhile to study another form which does not develop gemmules.

Similarly the origin of scleroblasts during the development is known only in a few studied by Dendy (1926) and Sivaramakrishnan, (1951), who observed that the scleroblasts are formed by specialised amoebocytes and vesicular nucleated cells which form part of the larval interior.

Further, it was felt that the development of Indian sponges has, so far, received comparatively less attention than other groups and warrants a more intensive study. So far our knowledge of the development of Indian sponges is confined only to three species, namely *Callyspongia diffusa, Tedania nigrescens,* and *Hircinia* sp. (Sivaramakrishnan, 1951) and many features in their development are still obscure.

In view of its distribution in tropical as well as temperate waters, *Lissodendoryx similis* was chosen for the present investigation.

Material and Methods

Material used in the following study was collected from the Madras harbour and the Royapuram beach. A detailed description of *Lissodendoryx similis* has been given in an earlier paper

(Ali, 1956). The sponges collected from the harbour are found growing on the sidewalls of the boat basin 2-3 feet below the low tide mark and on the bottom of old boats lying in the boat basin. The sponges from the Royapuram beach are found on the lower sides of the rocks which are exposed during low tide.

The specimens were kept alive in the laboratory for varying periods extending from 2 to 7 days in large glass troughs. The water was aerated and also changed at frequent intervals. The sponges thus kept were able to remain in a healthy condition.

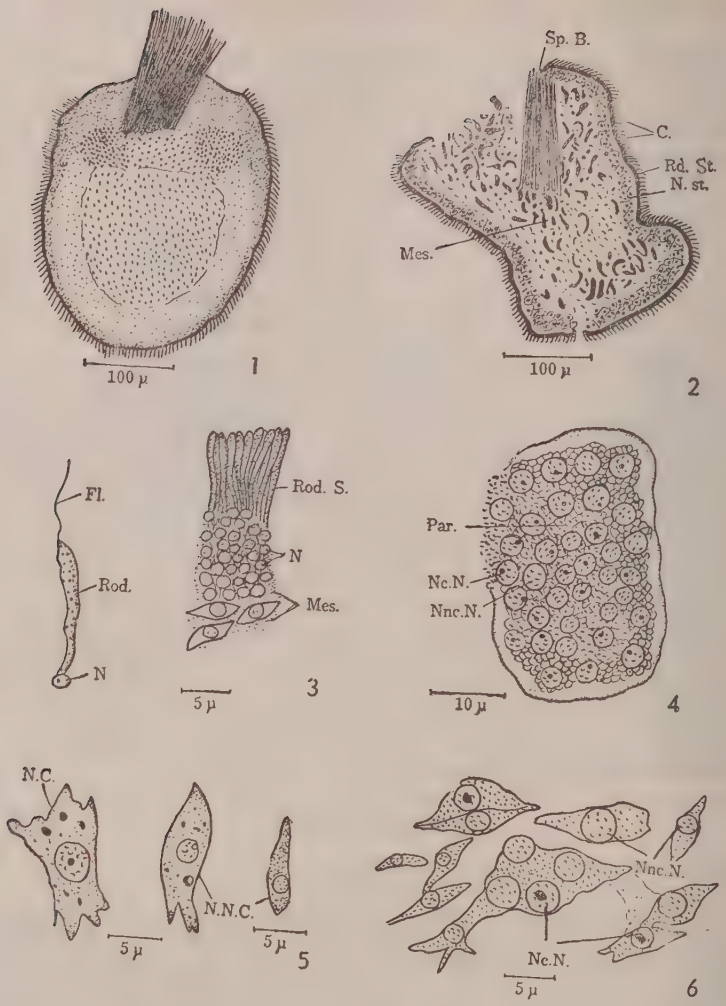
Nearly in all the cases the sponges extruded a large number of larvae 3-4 hours after they were brought from their natural habitat, probably due to the shock brought about by the handling and change in environment. The larvae liberated from the sponges were visible to the naked eye. They were collected and kept in finger bowls in which were glass slides coated with paraffin. The larvae settled on them and were scraped along with the paraffin coating and fixed. Bouin's fixative was found suitable. After clearing, the larvae were left only for, about 20 to 30 minutes in the paraffin bath. Sections were stained with Delafield's Haematoxylin and counter stained with Erythrosin. Hertwig's macerated fluid was used in the maceration preparations of larvae. Whole mounts of the larvae were stained with Delafield's Haematoxylin or Borax carmine. Sections were cut at 4 or 6 μ.

MOTILE LARVA

Description

Amongst the many cases of larval development in sponges which have been described only two species are known in which this takes place from asexual gemmules. These are *Esperella fibrexilis* (Wilson, 1891, 1894) and *Callyspongia diffusa* (Sivaramakrishnan, 1951). In other cases it has been assumed that development is from fertilised eggs.

Gemmules have not been found in *Lissodendoryx similis* nor did sections of the sponge show any indication of cell assemblages which are known to precede their formation. For this reason it has been presumed that larvae found emerging from the oscula were derived from fertilised eggs, although it has not been found possible to detect recognisable sex-cells. This is in accordance with the findings of Hyman (1940) on the development of sexual larvae in other sponges.



FIGS. 1 to 6.

FIG. 1. Surface view of free-swimming larva. Camera lucida drawing. FIG. 2. A median longitudinal section of the free-swimming larva showing the mesenchymal and epithelial cells. C. Cilia. N. St. Nuclear stratum. Rd. St. Rod stratum. Sp. B. Spicule bundle. Mes. Mesenchyme. FIG. 3. Epithelial cells (a maceration preparation from larva belonging to the same stage shown in FIG. 2. Fl. Flagella. Rod. S. Rod stratum. Mes. Mesenchyme. N. Nuclei. FIG. 4. A longitudinal section of the free-swimming larva passing through the median plane. Nc. N. Nucleolate nuclei. Nnc. N. Non-nucleolate nuclei. Par. Paranchyma. FIG. 5. Mesenchymal cells (a maceration preparation) from larva belonging to the same stage shown in FIG. 2. N. C. Nucleolate cells. NN.C. Non-nucleolate cells. FIG. 6. Cellenchyma cells from the larva at the same stage as shown in FIG. 2, (a maceration preparation). Nc. N. Nucleolate cells. Nnc. N. Non-nucleolate cells.

Larvae were obtained only during August to February. The larvae at the time of liberation from the sponge are white in colour, oval in shape, ciliated throughout (Fig. 1) measuring about 0·400 mm. by 0·250 mm. in size. The posterior end of the larva is broader than the anterior. Whole mounts and sections taken through the larva show that a number of spicules are not seen in the living larvae because of their opacity. The larva has all the features of a stereogastrula. Similar larvae of *Esperia lorenzi, Esperella fibrexilis,* and *Mycale syrinx* have been described by Maas (1892) and Wilson (1891, 1894, 1935). From the descriptions given by these authors of the larvae they had studied it was evident that the larvae liberated from *Lissodendoryx similis* are stereogastrula. However the larvae obtained by these authors measured 1·0 mm. and 0·650 mm. respectively. It is possible that these large larvae were liberated later though, these authors have not given details regarding the stage when they were liberated.

Immediately after liberation the larvae start swimming up towards the surface as in the case of *Esperia lorenzi* (Maas, 1892, 1893) and *Mycale syrinx* (Wilson, 1935) but start moving towards a corner of the trough which is farthest from light. After a short while a number of larvae are seen to lie in a group in that corner. The negatively phototropic nature of the larva is clearly seen when the sponge which liberated the larvae is kept in a well lit part of the trough and the larvae coming out are observed. Such larvae swim up and then start moving away from the light.

A few of the larvae behave differently from the majority. These larvae immediately after liberation swim with the rest in the body of the water and later do not retreat the surface away from light but ascend vertically to the surface with a rapid motion and either break into bits when they touch the surface or become adherent to the surface and undergo subsequent changes, epithelial layer becomes flattened circular and brownish. Such larvae may live for many days but undergo no further development. Eventually, they die. Abnormal instances have been observed by Wilson (1935), who stated "A curious type of behaviour is not infrequently exhibited by the motile larva, which immediately after discharge from the adult rises quickly to the surface, flattens and shivers into scattered cells and fragments as if torn by some osmotic force that induces a real explosion". He also stated that a few larvae get attached to the surface in a manner similar to that shown by the larvae of *Lissodendoryx similis* as described

earlier. The rest of the larvae swim about away from light for about twenty hours and then gradually sink down, probably weighted by the spicular load.

Internal Structure of the Free-Swimming Larva

Freshly liberated larvae were sectioned up to the stage when they sank to the bottom twenty hours later. These sections show an outer layer of cells forming the epithelium and inner mass, the mesenchyme, containing a number of tylotes, styles, sigmas and isochelae.

Megascleres:—It is observed that almost all the megascleres which are present are tylotes. Rarely, one or two styles are met with and it is of interest to note that the styles which are present in large number in the adult are poorly represented in the larva. The tylotes and styles are of the same size as those seen in the adult. These are seen to converge in bundles towards the spicular (posterior) pole of the larva (Fig. 2). The spicular heads of the tylotes are rounded as in the adult. Similar observations have been made in the case of *Esperia lorenzi* (Maas, 1892-1893) and *Mycale syrinx* (Wilson, 1935). The tylotes are slender with prominent heads and measure about 0·156 mm. to 0·182 mm. by 0·004 mm. to 0·0054 mm. and their heads are about 0·0081 mm. in diameter.

The styles are thicker than the tylotes and are bent towards the blunt end, which is rounded. The spicules terminate abruptly in sharp, pointed ends. The styles measure 0·176 mm. to 0·156 mm. by 0·0054 mm. to 0·0081 mm. and are present in very small numbers in the larva.

Microscleres:—Isochelae and Sigmas are present in the larvae. The larval Isochelae are of the same size as those seen in the adult sponge, but the Sigmas are slightly larger in the larvae. The Isochelae are bent in the middle and have tridentate ends, which are identical with one another. The three teeth forming the end plates are of the same size and appearance. The Isochelae are present in large number and are scattered in the body of the larva. They measure 0·028 mm. to 0·031 mm. The sigmas which are very few in number are 'C' shaped and are of uniform thickness but are much larger in size than those present in the adult. The larval sigmas measure 0·024 mm. to 0·034 in size.

The microscleres are scattered all over the body of the larva. The larval tissue contains more spicules than in the adult as may be seen from a comparison of two equal sized parts of the larva and adult.

The outermost layer of the larva (Fig. 2) composed of slender and very much elongated cells is referred to as the epithelium. Delage (1892, 1899) referred to it as the ectoderm. Wilson (1935) called it epithelium, since the term "ectoderm" denotes the outer layer of cells in higher animals originating from the epiblast of the embryo and does not seem appropriate to describe the outer layer of the sponge larva which undergoes a change in position at the time of metamorphosis.

In maceration preparations it is seen that a normal epithelial cell consists of an outer portion (Fig. 3) which is referred to as the rod and an inner thread-like portion, the fibril, which terminates in the nucleus. The rod and the fibril are granular. In a section of the larva it is seen that the cells are compactly arranged and are pressed together, hence it is difficult to determine the structure of an entire epithelial cell from a section. Only in a maceration preparation is it possible to study an entire epithelial cell composed of the granular rod, fibril and nucleus. The granules seen in the macerations are larger than those seen in sections, possibly due to the effect of the acetic acid in the macerating fluid. The cilium when it is present is very long, but in some cases is broken (Fig. 3).

In all the maceration preparations the cells seldom appeared complete. Usually the fibrillar part gets separated from the rod part. When epithelial cells are examined in compact groups (Fig. 3) as seen in sections of the larva, they seem to be separated by spaces between one another. The cells vary markedly in length and nuclei join together to form the nuclear stratum which appears as a thick layer. An average epithelial cell, from base to cilium (including the nucleus) measures from 38 to 14 in length. Wilson (1935) also notices such variations in the length of the epithelial cells in the larvae of *Mycale syrinx*.

Just as the nuclei of the epithelial cells form the nuclear stratum, the rods also form a compact layer (Fig. 2), known as the rod stratum. Even though the rods are closely packed they are separate from one another, in a cross-section it is observed that the rods are circular in outline. Cilia are borne at the extreme

outer ends of the rods which are irregular, rounded or pointed. The epithelial layer is not uniform in thickness in all parts of the larva (Fig 2). Towards the spicular end it decreases in thickness and the line of demarcation between the rod and nuclear strata becomes indistinct.

Although, the inner tissue of the sponge larva has been referred to as the endoderm by Delage (1892), Wilson (1935) pointed out that this tissue gives rise to the epidermis and endodermal membranes during metamorphosis and so referred to it as the mesenchyme or parenchyme. Observations similar to those of Wilson's were made by Weltner (1907), Wilson (1910) and Wilson and Penney (1930). The inner of the two layers of the larva of *Lissodendoryx similis* is not compactly and uniformly arranged and consists of two differently arranged tissues. This tissue occurs between the various parts of the canal system in the adult sponge and is referred to in the following account as mesenchyme.

The mesenchyme consists of a gelatinous and transparent matrix, which is commonly referred to as the mesogloea. Hyman (1940) suggested that this might be of the nature of a protein. The cells of the mesenchyme are arranged in the mesogloea. Hyman (1940) observed that, if in the mesenchyme the number of cells is great in the mesogloea, thus showing a packed appearance, it is referred to as the parenchyme. (Fig. 4) on the other hand, if the proportion of the mesogloea far exceeds that of the cells embedded in it, it is termed collenchyma (Fig. 6). The collenchymatous cells have large nuclei with the bulk of cytoplasm on one side so as to make the nuclei bulge out laterally (Fig. 5).

Some of the mesenchymal cells do not contain nucleolate nuclei and these are referred to as the non-nucleolate cells (Fig. 5). During metamorphosis these migrate to the surface and form the epidermis. Some cells are observed to cover the spicular pole and, though, these are very similar in appearance to the mesenchymal cells, are actually epithelial cells.

The condition described above undergoes changes noticed in later larvae. Here the parenchymal region contains a number of nuclei scattered about uniformly and the tissue between them has a fine reticular structure composed of minutely granular strands (Fig. 4). The cytoplasm is uniformly distributed all over the parenchyma and is not dense around the nuclei. Rarely, however,

the nuclei, especially the nucleolate ones, are surrounded by dense, granular aggregations of cytoplasm thus imperfectly marking out cell bodies, which remain interconnected. Usually the tissue ranges between the two extreme conditions described above.

Two kinds of nuclei are met with in the parenchymal tissue, which are referred to as nucleolate and non-nucleolate nuclei (Fig. 5). Wilson (1935) observed that in the larvae of *Mycale syrinx* the granular nuclei migrate to the surface at a later stage and become epidermal nuclei and in the present investigation also it was observed that some of the granular nuclei were near or actually on the surface. The larger nucleolate nuclei together with the cytoplasm have been referred to as archaeocytes by Maas (1890, 1891, 1892, 1893, 1896) and Delage (1890, 1891, 1892, 1893; 1899). In the present study it was observed that the granular non-nucleolate nuclei are more numerous than the nucleolate one. A few larval epithelial nuclei are seen beneath the nuclear stratum in the parenchyma and Wilson (1935), who made similar observations, suggested that they might have been found there due to early immigration, indicating the onset of metamorphosis. It is observed that some non-nucleolate nuclei are present in the nuclear stratum and the cytoplasm around them is not marked off from the tissue in which the epithelial nuclei lie. No cellular inclusions were observed in the larval mesenchyme as has already been noted by Maas (1892), Wilson (1894, 1935) and Sivaramakrishnan, (1951) in the case of the larvae of *Esperia lorenzi, Esperella fibrexilis, Mycale syrinx* and *Callyspongia diffusa.*

The cells covering the spicular pole are syncytial and have granular reticular non-nucleated nuclei. This layer is separated from the parenchyma by spaces, which are traversed by the processes extending between the parenchyma and the surface cells and crossed by the microscleres aggregated at this pole. Overlying this region, cells which migrated from the mesenchyme and those derived from the epithelial nuclear stratum are present. These form a flat layer of cells in some places, although, they are mostly composed of broad cells which at the junction with the larval epithelium form rounded projections. Maas (1891, 1892, 1893) and Delage (1890, 1892) described the epithelial cells flat, but Wilson (1935) made observations which agree with those recorded here. Wilson (1935) attributed the projections to amoeboid activity.

The mesenchyme is very watery towards the anterior (antispicular) pole (Fig. 2). The cells in this region, which are clearly marked out, contain nuclei which are either nucleolate or non-nucleolate, the latter being more adundant. The Collenchyma is watery and the cells are arranged around spaces and are concentrated on the sides, i.e., adjoining the epithelial layers. In some larvae, the collenchyma is interrupted at the apical region. Wilson (1935), who noted similar features in *Mycale syrinx* suggested that this might be an indication of the onset of metamorphosis, which begins with the immigration of epithelial nuclei. It is observed that several epithelial nuclei with surrounding cytoplasm lie in the collenchymatous region in thick, but loose layers.

Sections through some of the larvae well illustrate the gradual onset of metamorphosis even in the motile phase of the larva. The rod structure of the epithelial layer is less distinct, the nuclear stratum becomes thicker on the antispicular side of the larva and a few nuclei appear to have become more superficial and have left the nuclear stratum to lie in the rod-stratum. In sections of what appeared to be a slightly older larva, the nuclei appear to have become discharged into the rod-stratum with a thin envelope of cytoplasm around them (Maas, 1892). Though Delage (1895) thought that these bodies were globules of excretory material Wilson (1935) conclusively proved them to be parts of parenchyme cells which migrate outwards and mark the beginning of metamorphosis.

In maceration preparations, a few epithelial cells as well as nucleolate and non-nucleolate cells of the mesenchyme, exhibiting the effects of the macerating are found. The cytoplasm granular and without inclusions. The cells swell and become bladder-like due to the absorption of the macerating fluid. The rods become separated from the rest of the cells in such a way that the nuclei are left adrift without cytoplasm. The mesenchyme cells become swollen to a greater extent than the epithelial cells, therefore they have very thin walls.

Bottom-living Larva

Twenty hours after liberation the larvae descend to the bottom of the vessel and move about for the next two or three hours. They then start rotating, keeping their posterior poles stationary and this phase lasts for about two hours. This kind of rotating

movement has been observed in *Esperia lorenzi* (Maas, 1892) and *Mycale syrinx* (Wilson, 1935). About 5 hours after sinking to the bottom, when the larvae stop spinning round, they lie flat on their sides at the bottom of the container for about fifteen hours before getting attached to the substratum.

The larvae are identical in all respects with the earlier stages except that the posterior end of the former is more flattened and spicule bundles are seen on the posterior side.

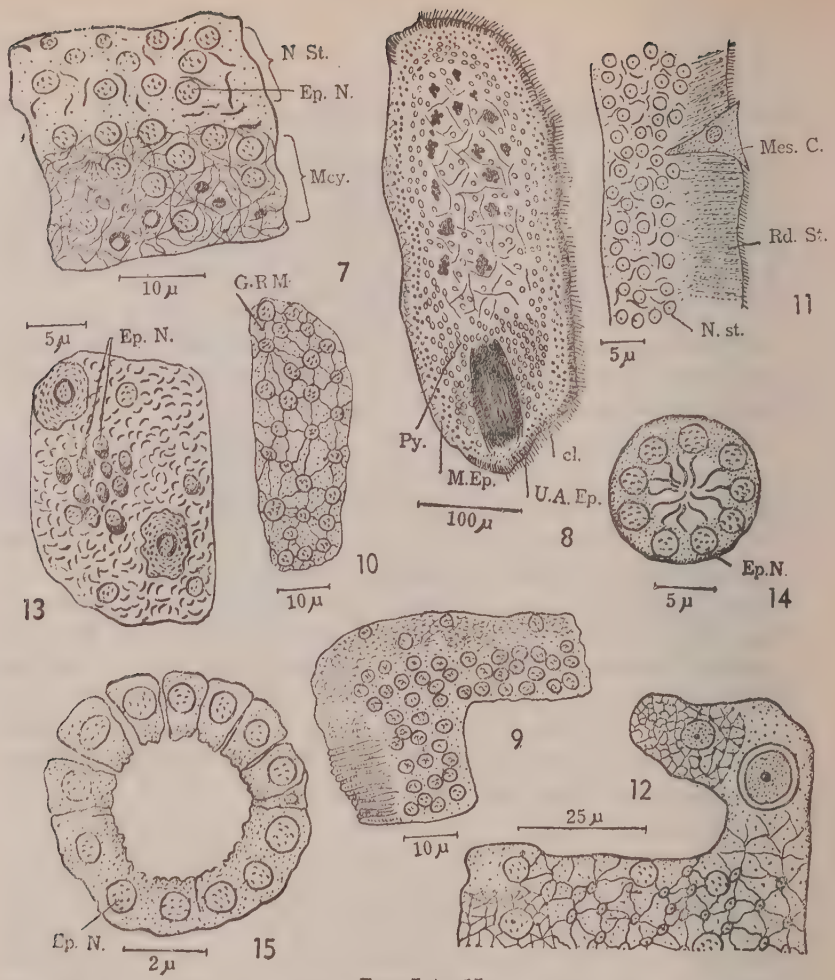
In sections of the larvae at this stage histological features which indicate evidences of metamorphosis are observed. The collenchymatous tissue of the larva which was continuous and large during the free-swimming phase gets distinctly subdivided through the formation of thick trabeculae, which may be considered a step in metamorphosis. At a later stage it is observed that the collenchymatous region of the larval interior becomes uniformly compact. Very rarely, however, it is observed that the process of immigration of the epithelial nuclei into the parenchyma, marking the beginning of the inversion of the outer layers commences in the bottom living larvae, although, normally this process takes place only after attachment.

Retarded Larva

Some larvae do not get attached to the substratum even twelve days after liberation from the adult sponge. These larvae get reduced in size and assume a globular shape. Similar observations have been made by Maas (1892, 1893) and Wilson (1935) in *Esperia lorenzi* and *Mycale syrinx*.

Evidences of metamorphosis as well as degeneration of certain larval tissues are seen in the sections of retarded larvae (Fig. 7). Although, the ratarded larvae still retain the larval epithelium, metamorphosis of the epithelium has taken place to some extent, in that the posterior end of the larva shows the presence of epidermal cells on the surface. Such features are seen to extend from the spicular to the anti-spicular end as has been noted in *Esperella fibrexilis* and *Mycale syrinx* (Wilson, 1894, 1935). In some of the retarded larvae the epithelium is not ciliated.

The epithelial nuclei are not very compact in the retarded larva in that the extension of the epithelial nuclei is grater than in the motile larva. The parenchyma is loosely arranged and some of the epithelial nuclei have moved into it. When the nuclear



FIGS 7 to 15.

FIG. 7. A part of the longitudinal section of the retarded larva shown in FIG. 17. N. St. Nuclear stratum. Ep. N. Epithelial nuclei. Mcy. Mesenchyme. FIG. 8. A section passing through a larva after attachment to the substratum showing evidences of metamorphosis. Cl. Cilia. M. Ep. Metamorphosed epithelium. U. A. Ep. Un-altered epithelium. Py. Parenchyma. FIG. 9. A section of the larva at the same stage as Fig. 8, showing the outer layers of cells at the surface of attachment. FIG. 10. A section of the larva in the same stage as in FIG. 9 showing the cells on the lower surface. G. R. M. Granular Nuclear Material. FIG. 11. A section of the larva in the 'first stage' of metamorphosis to show the mesenchymal cells passing to the surface. Mes. C. Mesenchymal cell. Rd. St. Rod stratum. N. St. Nuclear stratum. FIG. 12. Section of larva in the same stage as shown in the previous figure to show two degenerate nuclei. FIG. 13. A part of the longitudinal section of the larva as in FIG. 19 to show the grouping of epithelial nuclei in the reticular syncytium. Ep. N. Epithelial nuclei. FIG. 14. A section of the larva in the same stage as in FIG. 20 showing the epithelial nuclei forming the choanocytes. Ep. N. Epithelial nuclei. FIG. 15. The pro-chamber in the larval interior as seen in a longitudinal section of the larva in the fourth stage of metamorphosis.

stratum gets disorganised, some of the epithelial nuclei migrate to the exterior through the epithelial rod stratum. Wilson (1935) who made similar observations in *Mycale syrinx* suggested that this acceleration in migration might be due to cytoplasmic degeneration. It has been seen that in a motile larva some epithelial nuclei migrate to the surface and some into the larval mesenchyme. In a retarded larva this process is greatly accelerated and larger numbers of epithelial nuclei are seen to migrate to the surface and into the mesenchyme. This might be due to the degeneration of cytoplasm in the nuclear stratum and in the mesenchyme, which releases the epithelial nuclei and at the same time makes it easy for them to pass through the epithelial rod stratum and mesenchyme, which cease to be very compact.

The epithelial nuclei which migrate into the parenchyma, degenerate after a while and a number of small bodies, which are stained by nuclear stains are observed in the mesenchyme. Such degenerated epithelial nuclei, are in various stages of being absorbed by the syncytial mesenchyme.

The retarded larvae remain alive for a long period, usually four to five weeks, getting gradually reduced in size and it is observed that as they get older, they become brownish and eventually die.

First Stage

Nearly forty hours after liberation, the larvae begin to get attached. In most larvae attachment is on the side, while in a few it is on the spicular or anti-spicular poles. Four distinct stages in the metamorphosis of the larva can be distinguished. The first stage refers to a larva about twenty-four hours after attachment, the second after about forty-eight hours, the third after about seventy-two hours, and the fourth after nearly ninety-six hours.

Larvae attached by their sides: In a section of the larva after attachment it is seen that the epithelial layer on the side of its attachment shows evidences of metamorphosis, but such are not seen on the other sides. The cilia in the region of attachment are lost and rod-stratum is transformed into a granular reticular material without striations (Fig. 8). In this region the epithelial nuclei appear to lie much closer to the surface. That the granular reticular material is really derived from the transformation of the rod-stratum may be inferred from the position of this tissue

in relation to the nuclear stratum and its connexion with the rod-stratum, which persists as such on the lateral sides, unlike the side by which the larva is attached, does not show any evidences of metamorphosis.

The metamorphosis of the rod-stratum mentioned above is not complete or uniform (Fig. 9). On the upper side it still retains the rod-structure, but on the lower side it has become changed into the granular reticular structure. The rod structure on the lateral sides shows different degrees of transformation. In the middle region it is seen that it is neither rod structure nor granular reticular and on the lower part it is very granular but has not got the reticular structure. Wilson (1935) observed that in certain regions the nuclear stratum was curved inside and he attributed this to cytoplasmic currents constriction phenomenon in the syncytial cytoplasm. Similar observations were made by the author in the present investigation. In the granular reticular structure, delicate and finely granular strands which form meshes are observed. The strands are not uniform in shape and size and consequently the meshes are also not regular and are of different sizes.

The epithelial nuclei of the nuclear stratum start migrating towards the limiting membrane as the rod-stratum gets metamorphosed and some are actually in contact with the limiting membrane.

The nuclei of the epithelium, during the first stage of metamorphosis appear rounded and stain deeply. The presence of pycnotic nuclei is uncommon, however, such are present in the mesenchyme where they are not concentrated around the nucleolated nuclei as observed by Wilson (1935) in the case of *Mycale syrinx*. The pycnotic nuclei in the mesenchyme are found mostly in its outer parts.

A few mesenchymal cells with non-nucleolated nuclei are present in the epithelial layer and it is observed that some of them are on the point of breaking up. In a few instances non-nucleolated nuclei are found within the metamorphosed epithelium and these nuclei do not have any cytoplasm around them but lie in the syncytium freely. In the case of some which have distinct cell bodies a few granules are found in the cell body. After the above mentioned changes have taken place the outermost layer of nuclei has been distinguished as the epidermal nuclei of the

adult which forms a cellular tissue while some mesenchymal cells in the process of passing through the rod-stratum emerge as dead cells. The cellular tissue thus formed (Fig. 12) does not get integrated with the layer below it until the rods metamorphose into the granular reticular material. Wilson (1935) made similar observations in the case of *Mycale syrinx* and he suggested that these cell masses might have been shifted by protoplasmic currents set up by localised contractions in the reticular syncytium. The migration of cells towards the surface and to the epithelial layer may take place either by migration or cells in masses through the epithelium to spread on its outside where these cells later join the granular-reticulum into which the rods get transformed, or only the mesenchymal nuclei (Fig. 12) with or without surrounding cytoplasm may pass through the granular reticulum to just beneath the limiting membrane.

The metamorphosis of the epithelium commences at the spicular pole and gradually proceeds towards the antispicular pole. Metamorphosis of the rods takes place independently of the presence of mesenchymal cells or epithelial nuclei in the rod-stratum. It is observed that in regions where the larval epithelium has not metamorphosed there is no limiting membrane and the rods are separate from one another. The limiting membrane makes its appearance only when the rods get completely transformed into delicate granular reticular material. It appears that the limiting membrane is a part of the granular reticular material.

A large number of epithelial nuclei are seen in the mesenchyme of most attached larvae, but in a few cases; the nuclear stratum remains intact and only a few such may be found in the mesenchyme. The epithelial nuclei which are found in the larval interior lie in the syncytial tissue and do not possess well defined cell bodies.

The mesenchyme contains a large number of free cells. As in the motile larva the syncytial cytoplasm is not of uniform density and shows a number of spaces. It is seen that in this tissue, which is reticular, the cytoplasm does not condense around the nuclei but is evenly distributed with inter-connexions with other nuclei. Usually, three kinds of nuclei are present in this tissue, namely, nucleolate, non-nucleolate and those which have migrated from the epithelium. Delage (1892) thought that these epithelial nuclei may be carried into the interior along with the epithelial rods by the shortening and thickening of the epithelial

cells, resulting in their movement into the interior. Wilson (1935) disagreed with this view. The nuclei which have migrated from the epithelium lie in the syncytial tissue.

Wilson (1935) suggested that the occurrence of epithelial nuclei in the mesenchyme might be explained either by their migration from the nuclear stratum of the epithelium or by their having been left behind in that position during embryonic development.

The interior as mentioned earlier is syncytial with a few free or partially free cells as in the motile larva. A number of cavities in the collenchyma noted in the motile larva are present at this stage also. The tissue in this region towards the spicular end is more dense.

The reticular tissue becomes granular in a few places and is found to contain nucleolate nuclei, non-nucleolate nuclei and some immigrated epithelial nuclei. It is observed that the larger cavities have a lining which is formed by the cytoplasmic reticulum and in some places also by the denser cytoplasm where reticular structure is not clearly visible. The smaller cavities have no lining.

In the mesenchyme at this stage some of the migrated epithelial as well as the mesenchymal nuclei begin to undergo degeneration. The latter are larger, spheroidal in shape and homogeneous. The degenerate nuclei are digested by the reticulate syncytium. In the mesenchyme, membranes are present which appear similar to the outer limiting membrane. Such are formed by the rearrangement of non-cellular reticular strands. Wilson (1935) who made similar observations in the case of *Mycale syrinx* stated "The formation of membranes by a non-cellular system out of delicate reticulum, the strands of which presumably rearrange themselves in such a way as to accomplish that end, calls to mind Mrs. E. A. Andrew's generalization (1897, p. 28, et passim) that one of the characteristic properties of protoplasm, which with Butschli she conceives of as always alveolar, is the power to form thin films or pellicles at the surface of in the interior of the mass. She appropriately designates such structures as 'substance organs'. The membranes which I am here describing evidently fall in the category which she had made. Possibly also deLaubenfels (1932), has been observing something of the same kind. As I understand him he looks on the sponge he has been studying, one that is

related to *Mycale* (*Esperella*), as made up of cells and hyaline ground substance of a slimy, colloidal nature, probably poured out by the cells. To this he refers as a 'syncytial stuff' or 'slime', considering that it may be a form of cytoplasm, and perhaps comparable with 'Syncytium of Wilson and Penney (1930)'. This stuff forms a 'film over the surface and lining of the canals', and 'permeates the entire sponge'. It is 'a chief constituent of dermal membranes and canal linings'. By this meant, it would appear, that such membranes consist of the 'colloidal jelly' with 'cells clustered in it'. This point of view is strange to me. In the paper of Wilson and Penney (1939) and in the present paper syncytium is used in its usual sense as a protoplasmic complex distinct from the substance which lies in its meshes or otherwise bathes it. I am disposed to believe that deLaubenfels (1932) has been looking at delicate protoplasmic reticula similar to those which are described above".

Larvae attached by their anti-spicular poles: The histological details in the larvae that get attached by the anti-spicular poles are similar to the above. The points of difference are that the interior is more compact than in the previous case and the number of epithelial nuclei in the granular reticular material is more than that in the larvae that get attached by their sides.

Larvae attached by their spicular pole: In these larvae also the histological details noted are essentially the same as those in the first case. The difference lies in the larger number of the migrated epithelial nuclei in the larva. There are also more degenerate epithelial nuclei in the body of this larva than in the other two.

Second Stage

This stage is reached in about forty-eight hours after the attachment of the larva. At this stage it is more flattened due to the extension of the epithelium peripherally into the marginal membrane which is in the form of a very thin expansion. The marginal membrane extends over a considerable area of the substratum during this period. It is about ⅓rd or ¼th the thickness of the metamorphosing larva and is transparent. On the upper surface a few depressions are seen, and in these, the mesenchymal cells which are exuded from the larval interior are found. Sections of this larva in such regions reveal that the outer

layer of cells does not establish connexion with the rods underlying them until the latter get metamorphosed into granular reticular material.

Changes in the histological features (compared to those of the first stage) are seen. The outermost layer of the larva, which in the earlier stages is formed of the epithelium has completely metamorphosed into granular reticular material on the side of attachment and the lateral sides, but is in different stages of incomplete metamorphosis on the upper surface. When the position of the epidermal nuclei at this stage is compared with that seen in the previous stage it is observed that more of them are migrating towards the limiting membrane than in the earlier stage. Migration of the epithelial nuclei into the mesenchyme is also noted and as development proceeds they are seen to move in deeper. These nuclei do not have any cell bodies around them. Some of the mesenchymal cells which were in the process of migration towards the outer surface in the previous stage have passed through the epithelium and are seen to spread and form a thick layer over it. Maas (1892, 1895) and Wilson (1935) noted similar features in *Esperia lorenzi* and *Mycale syrinx*. This layer forms the epidermal cells.

At this stage the mesenchymal region of the larva becomes more compact but a number of small rounded spaces are still present and they seem to persist even in later larva. The compact region is found to be syncytial and to consist of continuous cytoplasm which is finely reticular or granular with nuclei which are either nucleolate, non-nucleolate or those which have migrated from the epithelium. Although a major portion of the syncytial tissue is reticular without cell boundaries a few collenchymatous cells are also seen. These cells are free and un-connected with the neighbouring tissue. These cells are either nucleolate or non-nucleolate and the former seem to be in larger number. The nucleolate cells show a number of inclusions. It is suggestive that such may have been derived from the degenerate epithelial nuclei and their digestion products, in view of the similarity in the staining reactions to those of the epithelial nuclei. Such an assumption finds support in the observation of similar structures by Maas (1892, 1893, 1895), Delage (1892) and Wilson (1894, 1935) in metamorphosing larvae of *Esperia lorenzi, Esperella fibrexilis, Tedania brucei* and *Mycale syrinx* which they identified as degenerate epithelial nuclei or their digestion products.

The syncytial cytoplasm into which the mesenchyme of the larva gets metamorphosed is in continuation with the granular reticular material into which the rods get transfromed. As observed in the preceding account the degenerate epithelial nuclei and their digestion products are seen only in nucleolate cells and Wilson (1935) who noted a similar condition in *Mycale syrinx* suggested that the pycnotic nuclei attract the nucleolate nuclei and these together form the nucleolate cells with pycnotic nuclei in them.

Third Stage

About a day after the second stage it is seen that the metamorphosing larva has spread further and is much thinner than in the previous stages. The marginal membrane is more extensive and is seen to cover a wider area than before. Surface views as well as sections of the larva at this stage show that the marginal membrane towards the very edge of the sponge is formed of only one layer of cells, whereas, towards the centre it is made up of two layers between which there is a thin layer of mesenchyme. In a few instances, mesenchyme is lacking between the two layers of marginal membrane. The appearance of this layer recalls strongly the 'Epithelial membrane" described by Wilson (1910, 1935) in *Stylotella* and *Mycale.*

Sections of the larva at this stage reveal that the larval epithelium has completely metamorphosed on all sides and the epidermal layer which is now fully formed is seen to cover the surface. This layer is very thin and is quite separate from the general syncytium. In some places epithelial nuclei which have migrated to the surface are seen between the limiting membrane and the syncytial material and these are flattened.

In a few metamorphosing larvae at this stage the epidermal layer and the syncytial material lying internal to it are seen to degenerate. Wilson and Penney (1930) noted similar instance in *Microciona.* Goette (1892), Maas (1892, 1893, 1899) Evans (1899) and Wilson (1935) also noted in *Esperia, Spongilla* and *Mycale* that the epidermis is formed as described above by the mesenchymal nuclei which have migrated to the surface.

Compared to the condition of the mesenchyme noted in the previous stage it is observed that at this stage it undergoes more marked changes. The tissue becomes more compact in the reti-

cular syncytium than in the marginal areas and in this feature it differs from the earlier stages in which the marginal tissue (epithelium) consisting of closely packed rods and nuclei was of a very compact nature whereas the mesenchyme consisted of loosely packed parenchymal and collenchymal cells. Only a few epithelial nuclei migrate to the surface at this stage, on the other hand a large number of them pass into the reticular syncytium and are found there scattered about irregularly. These nuclei are connected to each other by thin strands of the reticular syncytium which are evenly distributed except in a few cases where the cytoplasm of the reticular syncytium aggregates around the nuclei. These aggregations form cell bodies which are angular or rounded.

It is observed that during this stage the reticular syncytium has started breaking up into cells as in *Spongilla, Esperia, Lissdendoryx* and *Mycale* (Delage, 1892; Maas, 1892, 1893, 1895; Evans, 1899 and Wilson, 1894, 1935). It is also observed that the epithelial nuclei which have migrated into reticular syncytium appear to form groups of about eight or more. These at a later stage develop into choanocytes. Fig. 13 is from a longitudinal section of the larva at this stage in which the aggregation of the epithelial nuclei referred to is seen. In the reticular syncytium, nucleolate cells are more common than the non-nucleolate cells. In the former the pycnotic nuclei are found as inclusions, such may also be seen in the syncytium.

Fourth Stage

About twenty-four hours after the attainment of the stage described above, the larva passes into what may be called the fourth stage in its metamorphosis. At this stage the flagellated chambers and the canal system make their appearance and the sponge resembles in a number of features the adult.

Detailed descriptions and figures of this stage in *Esperella fibrexilis* and *Mycale syrinx* (Delage, 1892; Wilson, 1894, 1935) have been given. Delage (1892) stated that in *Esperella sordida* the reticular syncytium is compact throughout except in a few places where the cytoplasm aggregates around the nuclei and forms the flagellated chambers and canals. The reticular syncytium now becomes organized to form a cellular tissue by the aggregation of the cytoplasm around the nucleolate, non-nucleolate and the epithelial nuclei which form well defined cell bodies. These are connected with each other by thin strands except in a few

places where as in the previous stage the region was occupied by the reticular cytoplasm in which were found the epithelial, nucleolate and non-nucleolate nuclei, without cell bodies. Further, it is noted that in certain regions the tissue loses its compactness and breaks up into groups of cells which ultimately give rise to the flagellated chambers. The small spaces formed by the breaking up of the tissue of this region which are at first scattered about, later combine together to form the canals, the linings of which are formed by the non-nucleolate cells. Delage (1892), Maas (1892) and Evans (1899) noted similar stages in the formation of the canals, in the case of *Esperella sordida, Esperia lorenzi* and *Spongilla lacustris.*

Although the transformation of the larval epithelium is more complete than in the previous stages it is seen to persist in a few regions. But the formation of the epidermis is more complete at this stage. The epidermal cells show the same histological features noted in the previous stage. Strands connecting the epidermal layer with the mesenchymal cells appear, which are seen to extend specially to the nucleolate nuclei in the mesenchyme.

The nucleolate cells of the mesenchyme are present in large numbers and are interconnected by cellular strands. Both the nucleolate and the other cells in the mesenchyme are not uniform in size and contain inclusions which have been noted in the previous stage as formed of degenerate epithelial nuclei or their digestion products. It would appear that such inclusions are less abundant than in the previous stage. This might be due to the digestion of the inclusions by the cells concerned as has been suggested by Delage (1892) who noted similar features in *Esperella sordida.*

The non-nucleolate nuclei of the mesenchyme which do not migrate, to the surface from cell bodies are present, at this stage, in large numbers. These are connected by laminae instead of strands as in the case of nucleolated cells. Wilson (1935) suggested that the occurrence of such laminae might represent a stage in the formation of canal linings.

It has been pointed out that the epithelial nuclei migrating into the mesenchyme give rise to the choanocytes. At this stage the choanocytes are either scattered about or grouped together to form the flagellated chambers. The choanocytes are present in large numbers and are of different sizes (Fig. 14). Each has a

well defined cell body and the nucleus is always near the margin of the cell.

The flagellated chambers appear to be traversed by cytoplasmic strands. Wilson (1935) referred to these small flagellated chambers as pro-chambers (Fig. 15). The choanocytes at this stage do not develop flagella. The inter-nuclear strands seen in the previous stages probably form the threads inside the hollow of the pro-chambers. Such strands have been noted in other sponge larvae and may serve to transfer substances to and from the cells and also to hold them in their respective positions in the mesenchyme as suggested by Delage (1892) and Wilson (1935).

The choanocytes rarely divide and the pro-chambers increase in size by addition of free choanocytes drawn from outside the chambers. Delage (1892) and Wilson (1935) also observed similar growth of chambers in *Esperella sordida* and *Mycale syrinx*.

The choanocytes attain their normal shapes with collars and flagella at a later stage, although, a few of them seem to develop collars and others flagella or both. It would seem that the collars develop before the flagella appear. In addition to flagellated chambers described above, the mesenchymal region still contains the original syncytial reticulum comprising the nucleolate and non-nucleolate cells with their inter-connecting cytoplasmic strands. These are not visible in some preparations, due, possibly to the effect of fixatives.

In the mesenchyme at this stage there are also seen scleroblasts of different sizes, showing the development of spicules It is not clear from which of the cellular elements of the mesenchyme described above scleroblasts are derived, for in the preceding stages of development there appeared no indication by which the cells destined to form the scleroblasts could be distinguished. However, Sivaramakrishnan (1951) noted that in *Callyspongia diffusa* the scleroblasts are derived from the 'Vesicular nucleated cells', which correspond to the nucleolated cells of this form.

Discussion

The stage at which the larva is liberated from the parental sponge varies in different species and deserves comment. In structural features the larva of *Lissodendoryx similis* corresponds to the stereogastrula larva seen in *Esperella fibrexilis* (Wilson,

1894). Although in *Axinella cristagalli, Myxilla rosacea, Gellius varius, Chalinus fertilis, Hircinia variabilis, Euspongia officianalis, Esperia lorenzi* (Maas, 1890, 1891, 1892, 1893, 1895); *Esperella sordida, Spongilla fluviatalis* (Delage, 1890, 1891, 1892, 1893, 1899); *Spongilla lacustris* (Evans, 1899); *Mycale syrinx* (Wilson, 1935) it is not mentioned at what stage the larva is liberated, the diagrams given by these authors suggest a close resemblance of these larvae to those of *Lissodendoryx similis* and therefore probably were liberated at the same stage of development. The asexual larvae liberated from *Callyspongia diffusa* of Madras (Sivaramakrishnan, 1951) appear to be more highly organized than the stereogastrula larvae and are described as amphiblastula by Sivaramakrishnan (1951). Scleroblasts and spongoblasts developed only later on in the larvae of *Lissodendoryx similis* are already present in the larvae of *Callyspongia diffusa* which also have a pigment ring and indications of the onset of histogenesis. Thus it may be concluded that the asexual larva of *Callyspongia diffusa* is liberated in a more advance stage of development than the sexual larva of *Lissodendoryx similis.*

The duration of larval life in sponge varies in temperate and tropical forms and in sexual and asexual types of larvae. Generalisations are rendered difficult because of our ignorance of the development of a large number of species, however, we find that in *Lissodendoryx similis* of Madras, a tropical form, the sexual larva swims near the surface for nearly twentyfour hours and has a total free-swimming period of nearly forty hours. This is of interest because in temperate forms like *Esperella sordida* (Delage, 1892), *Esperia lorenzi* (Maas, 1892, 1893) and *Mycale syrinx* (Wilson, 1935) the sexual larvae swim near the surface for twenty hours. These authors do not say when the larva gets attached, but Sivaramakrishnan (1951) who studied the development of asexual larva of *Callyspongia diffusa,* found that the larvae are hatched not only at a much later stage but swim freely for only eight hours, whereas, in the temperate waters Wilson (1935) found the asexual larvae of *Esperella fibrexilis* swimming freely over twenty hours. It is well known that in some other groups of animals, tropical habitat abbreviates larval life. In sponges, it would appear that tropical habitat may not be the only factor responsible in such abbreviation of larval life, as may be found from the observation in *Lissodendoryx similis,* which, though occurring in the tropics shows a duration of free swimming larval life which agrees well with the allied species *Mycale syrinx,* occurring in the temperate seas.

The onset of metamorphosis is gradual or sudden and may take place early or late. Though the present form has a motile phase of about forty-eight hours, it develops metamorphic changes twelve hours after liberation. The nuclei of the nuclear stratum begin to invade outwards into the rod-stratum of the epithelium. This feature is also observed in the temperate form *Mycale syrinx* (Wilson, 1935) at about the same period. It is probable that the more rapid development of the asexual larva of *Callyspongia diffusa* when compared with *Lissodendoryx similis* from the same locality must be explained only as related to the asexual character of the larva and its origin from a multicellular well nourished body, namely the gemmule.

The larva of *Lissodendoryx similis* undergoes some changes in its external shape and sections reveal that some epithelial nuclei have started migrating into the mesenchyme. It is also seen that in many sponges such as *Esperella sordida, Spongilla fluviatalis* (Delage, 1892, 1893; Ganin, 1879; Goette, 1886); *Esperia lorenzi* (Maas, 1892); *Spongilla lacustris* (Evans, 1899); and *Callyspongia diffusa* (Sivaramakrishnan, 1951), metamorphosis commences only towards the end of the bottom-living phase. However, it is seen that in *Esperella fibrexilis* and *Mycale syrinx* (Wilson, 1894, 1935), the changes leading to metamorphosis, commence early in the free-swimming larval life as in *Lissodendoryx similis.*

It has been observed that an essential feature of metamorphosis is the passage to the exterior of the inner cells of the larva and a similar migration of the outer epithelial cells to the interior. This feature has been noted in most of the sponges with the exception of *Oscarella* (Maas, 1893), *Halisarca* (Meewis, 1939). However, in *Lissodendoryx similis* it has been noted that in the course of such inversion of the outer larval cell layers only the epithelial nuclei migrate into the mesenchyme as in *Mycale syrinx* (Wilson, 1935). In this feature it differs from the processes taking place in *Esperella sordida, Spongilla fluviatalis* (Delage, 1890, 1891, 1892, 1893, 1899), *Spongilla lacustris* (Evans, 1899), *Esperia lorenzi* (Maas, 1892, 1893) and *Callyspongia diffusa* (Sivaramakrishnan, 1951) in which the entire epithelial cells migrate into the interior where later some of them appear to take part in the formation of the choanocytes. Ganin (1879), working on *Spongilla fluviatalis,* thought that the outer layer of cells corresponding to the epithelial cells did not migrate into the interior but formed the epidermis of the adult. Goette (1886) who also studied the same sponge con-

sidered that the larval epithelial cells are discarded and the epidermis of the adult sponge develops from the outer layer of the mesenchyme. The significance of such variations is not clear, although it is suggestive that the larval cells whether they belong to the epithelium or mesenchyme appear to possess the potentialities to form one or other of the adult histological elements.

It is seen from previous work that the process involved in the migration of larval cells is not uniform or identical in all forms. For example in *Esperella sordida* (Delage, 1892) the epithelial cells are said to be engulfed by the mesenchymal cells, whereas in *Spongilla lacustris*, Evans (1899) observed that the immigrating epithelial nuclei formed plasmodial aggregations with the mesenchymal cells and separated again on reaching the mesenchyme. A similar observation has been made by Sivaramakrishnan (1951) in *Callyspongia diffusa*. Noldeke (1895) observed that during the immigration of epithelial cells in *Mycale* and *Spongilla*, they undergo destruction. In *Lissodendoryx similis* although the migration of the epithelial nuclei is clearly seen from an examination of sections of the larva in different stages, the means by which such movement inwards is effected is not clear. However, it was noted that in larval mesenchyme spaces appear preparatory to the migration of the outer epithelial nuclei.

In the larva of *Lissodendoryx similis*, the mesenchymal cells migrate to the surface to form the adult epidermis as noted in several other sponges such as *Esperella sordida*, *Spongilla fluviatalis* (Delage, 1892, 1893), *Esperella fibrexilis*, *Mycale syrinx* (Wilson, 1891, 1894, 1935), *Spongilla lacustris* (Evans, 1899) and *Callyspongia diffusa* (Sivaramakrishnan, 1951). The exceptions are *Oscarella* (Maas, 1893) and *Halisarca* (Meewis, 1939), in which such migration of mesenchymal cells to the surface has not been observed. In such forms it has been suggested by the authors who studied them that the epithelium which constitutes the outer layer of the larvae forms the adult epidermis.

The mesenchyme of larva which is a cellular tissue before metamorphosis, breaks up into cytoplasm and nuclei during late larval stages and consists of a reticulum in which the nucleolate, non-nucleolate and immigrated epithelial nuclei are seen to lie. The reticular cytoplasm is seen to connect these nuclei. Later, during metamorphosis, this reticular syncytium loses its reticular nature and the cells which are reconstituted form the choanocytes, canals and their linings. A similar mode of formation of the reti-

cular syncytium has been noted in *Spongilla lacustris* (Evans, 1899) and *Mycale syrinx* (Wilson, 1935). In *Spongilla fluviatalis* (Delage, 1893) the larval mesenchyme is said to break up into rounded, polygonal cytoplasmic masses. In the case of *Esperella sordida* (Delage, 1892) the mesenchymal cells as well as the immigrated epithelial cells are said to gather cytoplasm around them and give out pseudopodia which eventually unite and give rise to a reticular syncytium. Wilson (1935) considered that the diagrams given by Delage (1892, 1893) in the case of *Esperella sordida* and *Spongilla fluviatalis* were similar to those of *Mycale syrinx* and he suggested that Delage interpreted them in a different way, probably, due to the use of lower magnification.

In *Callyspongia diffusa* (Sivaramakrishnan, 1951) it is apparent that a reticular syncytium similar to that described in *Lissodendoryx similis* is not formed. It has been observed that the immigrated epithelial cells and the mesenchymal cells which did not migrate to the surface, remained as well defined invidual cells. Similarly in *Halisarca* Meewis (1939), the cells of the larval mesenchyme remain as well defined cells, without forming a reticular syncytium.

In *Callyspongia diffusa* the details regarding the histological changes in the larva preceding the formation of collar cells have not been studied. However, it was pointed out that the immigrated epithelial cells gave rise, at a later stage to the choanocytes. The formation of choanocytes during metamorphosis of the sponge larva has been described by a number of authors and considerable variations have been noted. In *Halisarca* (Meewis, 1939) it was pointed out that choanocytes were formed from cells which constituted the larval mesenchyme, whereas in *Esperella sordida, Spongilla fluviatalis* (Delage, 1892, 1893) and *Esperia lorenzi* (Maas, 1892) the choanocytes were said to be formed by the grouping of the migrated epithelial cells. In *Spongilla fluviatalis* (Delage, 1893) some nucleolate cells were also found to form choanocytes. It would appear that the choanocytes may arise either from the larval epithelial cells or mesenchymal cells in different sponge groups.

The formation of choanocytes in *Lissodendoryx similis* appears to be a variant of the type met with in *Esperella sordida* (Delage, 1892) and *Callyspongia diffusa* (Sivaramakrishnan, 1951). For unlike in the latter the choanocytes is formed not by

the entire epithelial cell but only by the nucleus together with a portion of the cytoplasm of the inner reticular syncytium which form a cell body. Therefore, it is clear that in *Lissodendoryx similis* both the mesenchymal as well as the epithelial parts of the larva are involved, in the formation of choanocytes. In the above features it agrees with the condition noted in *Mycale syrinx* (Wilson, 1935) and *Spongilla lacustris* (Evans, 1899).

The formation of the lining of the canals in *Lissodendoryx similis* in which the non-nucleated nuclei, which do not migrate to the exterior take part, is similar to that known to occur in *Esperia lorenzi* (Maas, 1892), *Spongilla lacustris* (Evans, 1899), *Esperella fibrexilis, Mycale syrinx* (Wilson, 1891, 1894, 1935) and *Callyspongia diffusa* (Sivaramakrishnan, 1951). It is seen that in all these forms as well as in *Lissodendoryx similis* a feature noted is that nucleolate nuclei get integrated with choanocytes in the process of formation of canals. That such a mode of formation of lining of canals is not universal is noted from the observation of Delage (1892) that in *Esperella sordida* lining of canals is formed by cells which have migrated from epithelium. The nucleolate nuclei of the mesenchyme in such forms do not take part in the formation of lining of the canals. From the above observations it may be inferred that in the formation of choanocytes as well as that of the lining of canals either the cells belonging to the mesenchyme or those derived from the epithelium may take part. The significance of the formation of these structures from the epithelial elements in some forms and from the mesenchymal elements in others is not known.

Summary

1. Previous work on the development of sponges is briefly discussed with reference to the difference between asexual and sexual larva, origin of choanocytes and of scleroblasts.

2. The liberation and structure of the free-swimming larva of *Lissodendoryx similis* are described in detail and compared to what is known of the free-swimming larvae of other forms.

3. Brief notes are given regarding the free-swimming larvae during their bottom-living phase. Retarded larvae are commented upon.

4. The four stages of development after the attachment of the larva are described with details of the changes undergone.

5. The stage at which the larva is liberated, the duration of free-swimming phase, the onset of metamorphosis in *Lissodendoryx similis* are discussed in relation to previous work.

6. The reversal of cell layers, the nature of the mesenchyme cells, the formation of reticular syncytium, the formation of choanocytes are briefly discussed together with other histological details.

ACKNOWLEDGEMENTS

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Commentaria Herbarii

Herbarium Collegii Presidentiae Madrasensis

5. Petiolar Anatomy and Subgeneric Classification of the Genus *Alangium*

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AND

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ABSTRACT

The petiolar anatomy of the species of *Alangium* is investigated. The vascular structure of the petiole is specific to each of the four subgeneric sections recognised by Bloembergen, and there is no overlap of the patterns among the subdivisions of the genus. The present study illustrates the fruitfulness of exploring anatomical characters towards identification, subgeneric segregation and systematic placing of the taxa even within a genus.

It is appropriate to recollect at the outset that in the well-known standard systems of angiospermous classification, criteria pertaining to structures other than the flower have been utilised in the classification of taxa that are smaller than families. The outstanding examples of such precedence will become evident when Die natürlichen Pflanzenfamilien of Engler and Prantl is perused. The recognition of the tribes within the family Acanthaceae has been accomplished on the basis of pollen morphology (Lindau, 1895). There has been a taxonomic precedence for utilising characters of the secondary xylem in delimiting the divisions of the family Piperaceae (Engler, 1899), Icacinaceae (Engler, 1893; Engler and Prantl, 1896), and Monimiaceae (Pax, 1889). Further investigations on the latter two families (Bailey and Howard, 1941 a, b, c and d on the Icacinaceae and Money, Bailey and Swamy,

1950 on the Monimiaceae) clearly indicate the role of anatomical data in the identification, recognition and systematic placing of taxa within the respective families. Marco's (1935) investigation on the secondary xylem of the Rhizophoraceae, although of a preliminary nature, contribute very significant information particularly in regard to the limits of the sub-divisions of the family. Likewise, there appears to be suggestive evidence for a revision of the Dipterocarpoid representatives with the aid of information obtained through a study of the petiolar structure as indicated by Brandis (1894). These instances bespeak the advisibility and often the necessity of obtaining data from structures and organs of the plant body other than the flower. The object of the present investigation is to extend the anatomical approach in the taxonomic delimitation of the species within the genus *Alangium*.

According to Bloembergen (1939), the genus *Alangium* consists of eighteen species, all of them occuring in the Old World and one species having an extended distribution in South Africa. Six species are confined exclusively to the Northern Hemisphere and two are essentially Southern; the remaining species extend on either side of the equator. Some of the species are rather restricted in geographical range while others display a wider area.

Bloembergen recognises four sections under the genus. In general, the sections Rhytidandra and Conostigma are essentially of South Eastern Asiatic distribution, while the sections Angolum and Marlea extend further into the northern latitude and also westwards to Africa. It may be mentioned here that Bloembergen's segregation has been accomplished essentially by an analysis of exomorphic characters. His monographic study indicates the occurrence of an unusually wide range of morphological variability within the genus. It is this phenomenon that prompted us to examine the endomorphic characters.

The length of the petiole in each section of the genus varies between definite limits and the limits do not overlap in the sections. Thus, in Angolum, the average range is 5 to 12 mm., in Marlea 9 to 51 mm., in Rhytidandra 3 to 20 mm., and in Conostigma 8 to 27 mm. The following account concerning a study of nearly two thirds of the species of the genus, likewise, clearly indicates that the ranges of variability met within the structure of the petiole are distinctive to each section.

Section I. Angolum.

There appears to be two more or less distinct types of petiolar vasculature, although both types conform to one and the same norm. In *A. salvifolium,* the petiolar vasculature occurs in the form of hollow cylinder, which is somewhat flattened on the adaxial side, appearing in a transection in the form of an unbroken ring (Fig. 1). In *A. longiflorum,* the vasculature tends to be rather eustelic, in which the individual strands are distinct, particularly in regard to the abaxial sector; the adaxial part, however, is made up of generally three strands which unite laterally to constitute a strap shaped structure (Fig. 2).

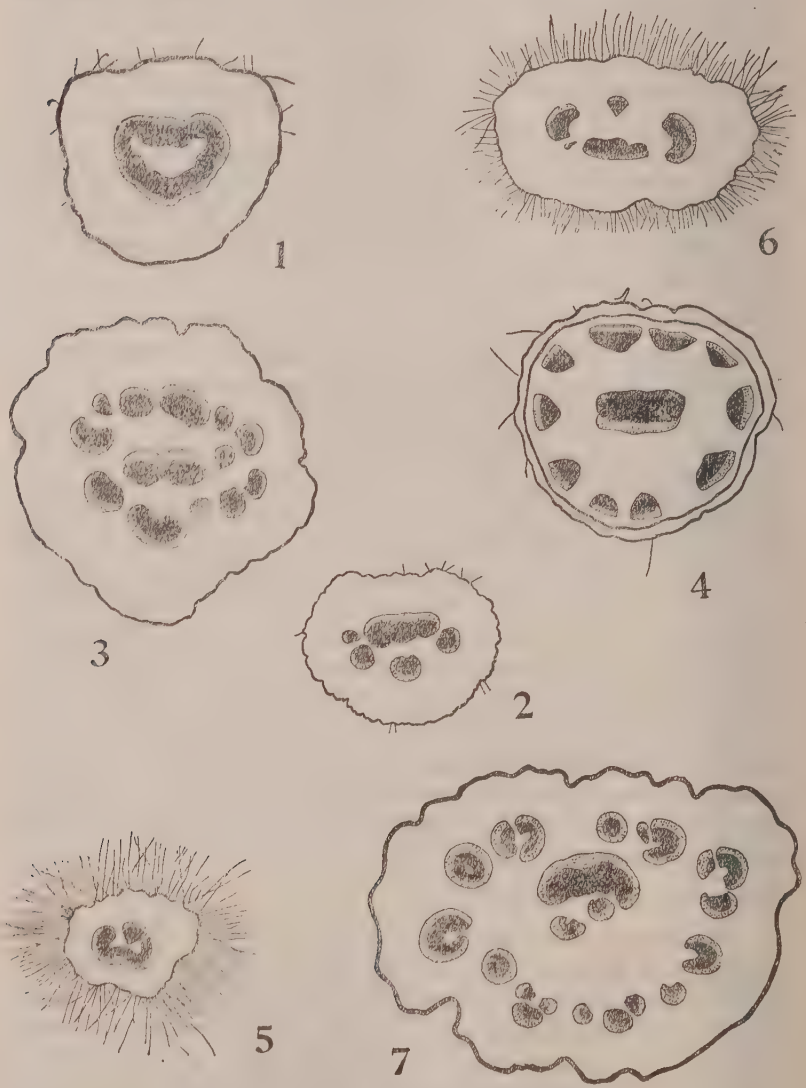
Section II. Marlea.

This section comprises of eight species. In *A. rotundifolium, A. griffithii* etc., the vascular strands become arranged in the pattern of a ring, each strand being separated by conspicuous interfascicular parenchyma which is continuous centripetally into the pith and centrifugally into the cortex. The centre of the pith, however, is occupied by a strap shaped strand of vascular tissue; the protoxylem of this strand is abaxially oriented (Fig. 3). The second type of petiolar vasculature is exemplified by *A. platanifolium, A. kurzii* etc. The arrangement of the vascular bundles in these representatives also conforms to the eustelic pattern. However, the ring of bundles is situated relatively nearer to the periphery than in the former kind (compare Figs. 3 and 4). Also, the individual peripheral bundle of the second group is typically collateral (Fig. 4), in contrast to that of the first kind where it is more or less tending to become concentric (Fig. 3). The occurrence of a strap shaped strand in the pith, however, is shared by both patterns.

Section III. Rhytidandra.

The petiolar vasculature in the representatives of this section presents two patterns. In one kind, the vascular tissue occurs in the form of a horse shoe shaped arc which constitutes the major segment; the free arms slightly converge towards each other thereby leaving an opening on the adaxial side. This opening is occupied by a small vascular strand with endarch orientation. Such is the situation seen consistently in the subspecies *villosum, vitiense, polysomoides, tomentosum,* etc. (Fig. 5). The second pattern occurs in the subspecies *ferrugineum.* Here, the vascular tissue assumes the form of four or five equidistantly placed discrete

strands arranged in the form of a ring, very much like an eustelic cylinder (Fig. 6).



TEXT FIGURES 1-7. Transections of petioles.

All figures are oriented with the adaxial side of the petioles pointing towards the top of the page. FIG. 1. *A. salvifolium;* FIG. 2. *A. longiflorum;* FIG. 3. *A. rotundifolium;* FIG. 4. *A. platanifolium;* FIG. 5. *A. villosum;* Ssp. *tomentosum;* FIG. 6. *A. villosum* Ssp. *ferrugineum;* FIG. 7. *A. ridleyi;* All figures × 22. The drawings are oriented with the adaxial surface of the petioles towards the top of the page.

Section IV. Conostigma.

In contrast to the petiolar structure described for the above three sections, that of the present section is characterised by the possession of two concentric steles. The outer stele consists essentially of deeply cresent shaped vascular bundles amongst which some are amphicribral. The inner stele consists of decidedly a smaller number of vascular bundles, the number, size and shape being subjected to considerable fluctuation. Generally, the adaxial segment of the inner stele is a larger one, while the abaxial vasculature consists of two or three smaller strands. (Fig. 7).

A summary of the investigation may be presented in the form of a key as follows:

Petiole monostelic

Medullary strand absent

Vasculature an unbroken ring

or

Constituted of an adaxial strap-shaped strand and of abaxial discrete strands .....Section I. *Angolum.*

Vasculature horse-shoe shaped open on the adaxial side, the opening being occupied by a smaller strand

or

Vasculature of discrete strands of varying number (3-5) arranged in a ring .....Section III. *Rhytidandra.*

Medullary strand present .....Section II. *Marlea.*

Petiole distelic, the two steles concentrically disposed

.....Section IV. *Conostigma.*

The above conclusions are thus in perfect harmony with the taxonomic procedure proposed for the genus (Bloembergen, 1939). The study also illustrates a rather close synchronization between the exomorphic characters in general and the anatomic characters of the petiole in course of phylogenetic modification. That such is the case in regard to several other endomorphic characters also will be shown in later contributions.

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Female Gametophyte and Endosperm of *Ophiorrhiza mungos* L.

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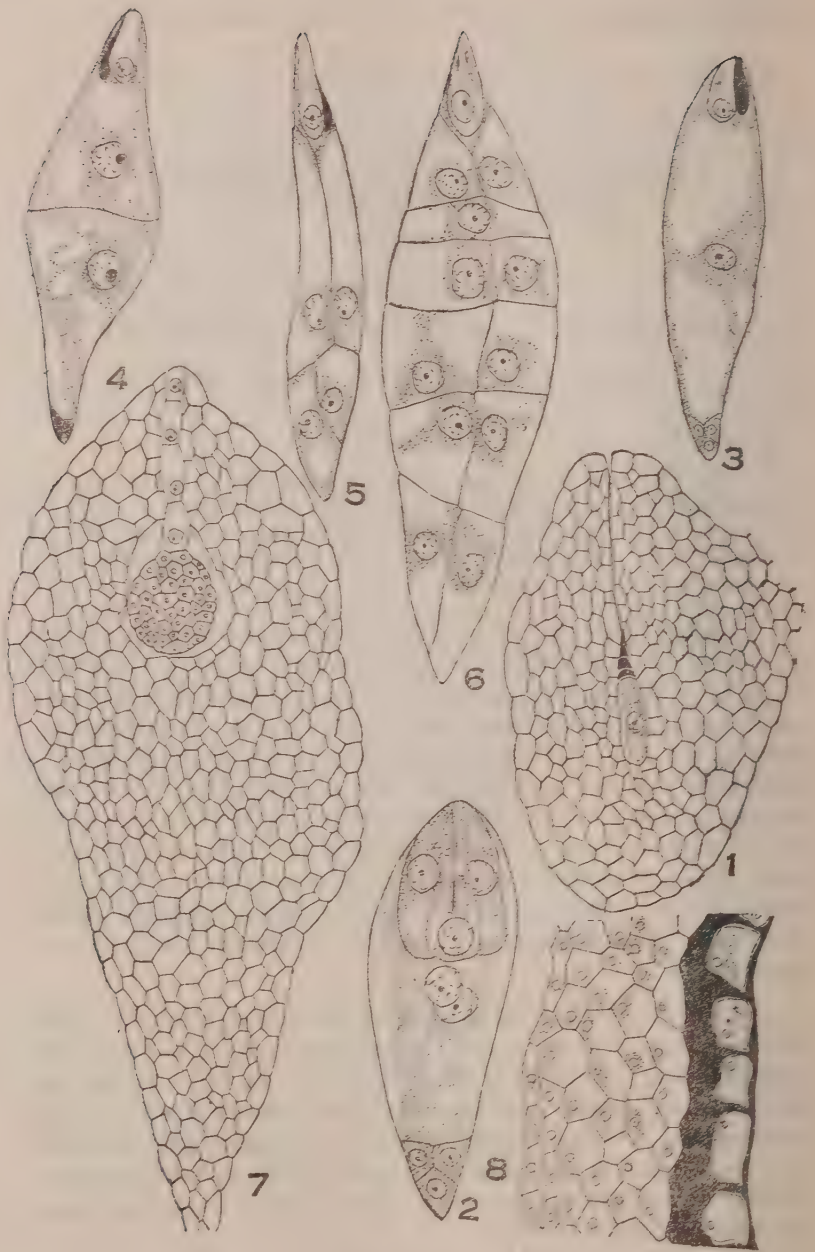
ABSTRACT

The development of the female gametophyte in *Ophiorrhiza mungos* L., conforms to the Polygonum type. Endosperm is *ab initio* cellular and in this respect the species contributes a significant exception to the other members of the family, wherein nuclear type of endosperm has been reported.

Although the rubiaceous taxa are underlined by a rather uniform type of flower structure, the family presents considerable range of variability both in regard to anatomical and embryological characters. In the construction of the ovule and in the development of the male and female gametophytes, important differences have been recorded amongst the taxa of the family.* It is significant to note however, that the endosperm has been recorded to be uniformly of the *ab initio* nuclear type, but for the suspected cellular type of endosperm reported for *Coffea* (Houk, 1938). This uniformity in endosperm development prompted Fagerlind (1937) to remark, "Das Endosperm ist bei den Rubiaceen durchegehends nuklear."

Ophiorrhiza mungos L., is an erect herbaceous plant with white flowers arranged in terminal dichotomous cymes. The flowers are actinomorphic, sympetalous, epigynous and perfect. Sepals are short, five-lobed and valvate. Corolla is funnel-shaped with five white lobes. The five epipetalous stamens alternate with the corolla lobes. Ovary is two celled with many ovules on axial placentas. Ovules are hemianatropous. Fruit is a coriaceous capsule dehiscing by two broad valves.

* For a summary of the more important embryological literature, see Ganapathy (1956).



FIGS. 1-8.

FIG. 1. Longisection of the ovule soon after formation of the tetrad × 540; FIG. 2. Mature embryo sac × 635. FIG. 3. Fertilised embryo sac × 635. FIGS. 4-7. Stages in the development of the cellular endosperm × 540. FIG. 8. A part of the seed coat × 540.

The nucellus is highly reduced comprising of a tiny protuberance arising from the placenta. This situation simulates the *Phyllis* type described by Fagerlind (1937). Archesporial cell differentiates in the hypodermal layer and functions directly as the megaspore mother cell. The remaining nucellar cells are ephemeral and are absorbed by the time of the formation of the megaspore tetrad. Due to the very early degeneration of the nucellar cells, the developing gametophyte appears as if abutting the single integument (Fig. 1). The megaspore mother cell divides and forms a linear tetrad of megaspores (Fig. 1) of which the chalazal megaspore develops into an eight nucleate embryo sac (Fig. 2) The egg apparatus consists of synergids with tapering apical ends and hemispherical bases (Fig. 2). The antipodal cells are three in number and degenerate only after fertilization. The behaviour of the antipodals does not conform to either of the types recognized by Fagerlind (1937). According to him, in the first type, the antipodals become swollen and often exhibit nuclear divisions. And in the second type, only the basal antipodal cell enlarges and acts as a haustorium. Raghavan and Rangaswamy (1941) have described a third type where the antipodals do not enlarge but degenerate long before fertilization. In *Ophiorrhiza* the antipodals retain their original size and number (Fig. 3) and show signs of degeneration only after fertilization. This feature is a distinct deviation from the patterns described for Rubiaceae.

The entry of the pollen tube into the embryo sac causes the destruction of one of the synergids. The other synergid persists for some time after fertilization (Fig. 3).

The division of the primary endosperm nucleus precedes that of the zygote. The first division is followed by a transversely oriented membrane separating the fertilised embryo sac into two chambers of equal size (Fig. 4). Subsequent longitudinal, transverse or oblique divisions of these chambers result in a mass of endosperm tissue (Figs. 5, 6, 7).

The most significant point in the embryology of *Ophiorrhiza* is the type of endosperm development. The intensive investigations of Lloyd (1902) and of Fagerlind (1937) reveal that endosperm is nuclear. The same type of endosperm formation is reported by the later workers also (see Ganapathy, 1956). Houk (1938) however reported a cellular type in *Coffea*, but noted that "the endosperm is not always strictly of the cellular type" as he found one embryo sac (fertilised) with free nuclei. Mendes (1941) in his study of

Coffea arabica found only free nuclear stages and clearly states that the "endosperm belongs to the 'Nuclear type'." Hence, with the exception of the report of Houk, the pattern of endosperm development in the Rubiaceae is nuclear. *Ophiorrhiza mungos* L., provides a significant exception. This situation emphasizes the necessity of a more detailed study of the other taxa of the family.

The mature seed coat is comprised of the remains of the integument. The epidermal cells develop excessive thickening of the walls along the inner tangential faces and become highly refractive (Fig. 8).

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A New Woodborer, *Bankia (Neobankia)* Denticuloserrata from Madras

BY

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ABSTRACT

A new woodborer, *Bankia (Neobankia) denticuloserrata* sp. nov. is described.

Introduction

In the course of a detailed survey of the marine borer fauna of the Madras coast, six specimens of the Genus *Bankia* were collected on 12th November 1955, which could undoubtedly be assigned to the Sub-genus *Neobankia*, since, the cone-in-cone structures were covered by a thin membrane denticulate at the free margin. On closer examination, these specimens were found to differ from the seventeen valid species included in this subgenus, of which, only one, *Bankia (Neobankia) lineata* Nair has been recorded from India before, and are hence referred to a new species *Bankia (Neobankia) denticuloserrata.*

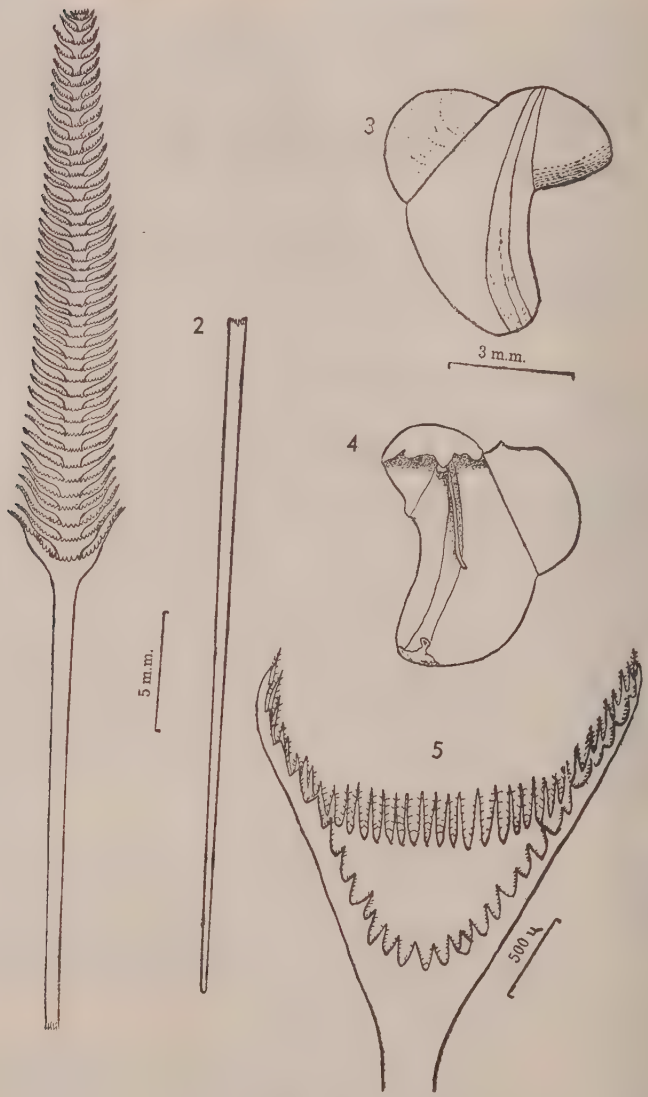
Size

All the six specimens were more or less of the same size measuring 6·4 cm.

Shell length—6 m.m.,	Shell height—6 m.m.
Pallet length—75 m.m.	Stalk of pallet—50 m.m.
Diameter of blade—2 m.m.	

Shell

Shell globular and white. Extreme anterior part with a deep sinus and reflected callus with smooth surface. The rest of the anterior area is marked by 62 denticulated ridges spaced at regular intervals, with the ridges twice as thick as the space between them and the free-edges of these ridges bearing rows of denticles. The length of the anterior lobe is almost equivalent to its height. The anterior-median part is marked by closely arranged rows of strong



FIGS. 1-5.

1. Pallet—Entire Blade and part of Stalk; 2. Remaining part of Stalk; 3. Right Valve—outer view; 4. Right Valve—inner view. 5. Outer face of single cup of pallet.

and conspicuous denticles. The dental ridges of this lobe meets those of the anterior lobe at slightly more than a right angle.

The middle-median part of the shell extending from the umbonal region to the ventral knob, is depressed and is smooth. The posterior-median part is slightly wider than the anterior and median parts combined, and feeble lines of growth are clearly discernible.

Interior of the shell is smooth with a strong umbonal knob from which the apophysis extends for more than half the distance from the umbone to the ventral knob. The auricle extends over the posterior median part forming a narrow shelf.

Pallets

The pallet is of the cone-in-cone type, with joints clearly separated from one another and a long fragile cylindrical stalk which is twice as long as the blade. The blade consists of distinct well-formed cone-in-cone structures ranging from 38-44 distinct cones in the specimens collected. The lateral borders of each cone are drawn out into slender processes and the intermediate space strongly denticulate by about 30 to 36 prominent denticles on each of the outer and inner margins. The inner margin of each cone is shallower with the denticles arranged at regular intervals. The outer margin is deeply concave and the denticles which are shorter and stouter are irregularly arranged. Each denticle bears on either side 5-8 fine serrated structures.

Siphon

The siphons are very long and conjointed up to the extremities. Both inhalant and exhalant siphons are equal in length, with the inhalant broader than the exhalant siphon.

Collar

Present at the base of the siphons.

Taxonomic Remarks

The Genus *Bankia* has been grouped into four subgenera, *Bankia* Gray 1840, *Neobankia* Bartsch 1921, *Bankiella* Bartsch 1921, and *Nausitora* Wright 1865. The subgenus *Bankia* is characterized by the pallet consisting of a series of cone-in-cone elements,

the distal edge of the cone terminating in a thin membrane fimbriated at the free margin and the lateral fimbriations forming long awn-like projections. The subgenus *Bankiella* is characterized by the pallet consisting of a series of cone-in-cone structures covered by a thin membrane which is neither fimbriated nor denticulated at the free margin but entire.

The subgenus *Nausitora* is characterized by the cone-in-cone elements not being entirely free at their distal ends but fused on the exterior surface where some shelly material and a thick periostracum cover the entire pallet. The subgenus *Neobankia* is characterized by the pallet consisting of a series of cone-in-cone structures covered by a thin membrane, which is denticulate at the free margin, and therefore the present form clearly belongs to this subgenus, *Neobankia.*

It appears that only about seventeen valid species have been recorded in this subgenus, of which, only one, *Bankia (Neobankia) lineata* Nair has been recorded from India before. The present form closely resembles *Bankia (Neobankia) lineata* Nair in the general features of the shell but differs from it in the possession of a pallet with about 38-44 well-defined and distinct cone-in-cone structures when the over-all pallet lengths is 75 m.m., in the possession of 30 to 36 prominent denticles provided with 5-8 fine serrated structures on either side on each of the inner and outer margin of each cone, in the cylindrical stalk being twice as long as the blade and in the number of the cutting ridges on the anterior lobe of the shell.

Hence the present form is treated as a species new to science, *Bankia (Neobankia) denticuloserrata,* and can be defined as follows:

Bankia with a linear pallet, the stalk of which is twice as long as the blade, the latter composed of 38-44 cone-in-cone elements when the overall length of the pallet is about 75 m.m., the free border of the cups bearing 30 to 36 denticles, each denticle bearing 5-8 fine serrated structures on either side, 62 denticulated ridges on the anterior lobe of the shell, and with a shell whose length is equal to its height having a well marked, thin, translucent auricle.

Types

The type will be deposited in the Indian Museum Calcutta. The paratypes will be in the Zoology Laboratory, Madras.

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Physiology of Digestion in *Bankia Indica* The Enzymatic Activity of the Digestive Diverticula†

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ABSTRACT

In the digestive diverticulata of *Bankia indica*, the presence of enzymes which hydrolyses carbohydrates such as starch, sucrose, glycogen, cellobiose, maltose, lactose, saw-dust, regenerated filter paper and gum arabic; proteins such as gelatine, fibrin, peptone and casein and lipidis such as methylacetate, lecithin solution and olive oil is indicated. The optimum conditions for the activity of the enzymes are described. It has been shown that the optimum is not affected by changes either in the duration of the experiment or in the hydrogen-ion concentration.

The results of the study on the different enzymes present, the conditions under which they operate and the end products obtainable through their action are discussed in relation to the food of this teredine *Bankia indica* and in relation to the findings of other workers.

Introduction.

Though the physiology of digestion, in a number of lamellibranchs has been studied (see Yonge, 1926, 1931, 1937; Vonk, 1937), yet that of the wood-boring teredines has not received much attention. Harrington (1921), found the extracts of the digestive diverticula of *Teredo* yielding reducing sugars, when treated with starch and saw-dust. Dore and Miller (1923), showed that about 80% of cellulose, and 15 to 56% of hemicelluloses disappear from the wood during its passage through the digestive tract of *Teredo navalis*, while Miller and Boynton (1926), and Boynton and Miller (1927), demonstrated the presence of cellulase which can form

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sugars from saw-dust and filter paper, in the digestive diverticula and its absence from the crystalline style of *Bankia setacea*. Recently, Lane and Greenfield (1952), have reported the presence of a cellulase enzyme system located in the general gut-wall "In the anterior third of the gut" of *Teredo*. They further showed (1953), that the cellulase activity of the post-caecal portion of the gut is approximately double that of the precaecal part. The role of the crystalline style in the digestive process of the ship worm has been recently reported by Nair (1955a, 1957).

Beyond this, we have no clear and detailed information regarding the nature, specificity and activity of the enzyme systems contained in the digestive diverticula. That the ship worm feeds on wood into which it bores is suggested by the fact that wood particles are found in the lumen of the specialised part of the digestive diverticula and in the caecum. The physiology of digestion in this highly specialised form would be of considerable interest in itself, as well as for comparison with the results obtained, with other lamellibranchs. Hence, a detailed systematic study of the active enzyme preparations of *Bankia indica* is attempted here, with special reference to, (a) the hydrogen-ion concentration of the medium, (b) duration of digestion, (c) temperature at which digestion is carried on, (d) the strength of the enzyme in the digestive mass and (e) the effect of substrate concentration on enzyme activity.

Material and Methods.

Since the digestive diverticula is small in these wood-boring molluscs a very large number of these animals had to be chiselled out of the blocks of timber in a living condition and the organs had to be separated as quickly and carefully as possible to obtain a sufficient quantity of enzyme in a fresh condition.

The digestive diverticula were washed in filtered sea water, dried between folds of clean muslin cloth, dehydrated and partially defatted by treatment with ice cold acetone (Sumner and Somers, 1947). The material was rapidly ground up in a clean glass mortar and repeatedly washed in acetone and filtered till the filtrate was colourless. The powder thus obtained was dried under a fan and stored in a clean bottle in a dessicator and kept at 3 to 4°C in cold storage. In all experiments 0·5 gm. of the powder was extracted with 200 ml. of distilled water for six hours at room temperature,

centrifuged and the clear centrifugate was used as the digestive extract.

The carbohydrase activity was studied using starch, sucrose, glycogen, cellobiose, maltose, lactose, filter paper; regenerated filter paper and also saw-dust from the timber in which the animals are found in nature, as substrates. The proteolytic activity was studied on proteins such as gelatine, fibrin, pepsin, and casein and for the lipolytic activity both natural and synthetic lipids were chosen as substrates.

Estimations of glucose were made in the first three experiments by Benedict's quantitative reagent (Bodansky and Fay, 1947) for the carbohydrase activity of the digestive diverticula. The reduction of dissacharides was detected by Barfoed's test (Yonge, 1924), as given in Bodansky and Fay (1947). In testing the diastase activity the method of Somogyi (1930), was preferred for estimation of the reducing sugars since the iodine-thiosulphate titration furnishes a sharp end-point.

The proteolytic activity of the enzyme preparations was followed by estimating the products of digestion by Srensen's formol titration method (Bodansky and Fay, 1947), while those of the digestion of fat were determined by the direct titration of the fatty acids liberated with standard sodium hydroxide solution, using phenolphthalein as indicator.

In all the enzymic experiments, the pH of the medium was controlled by suitable buffers such as Srensen's M/15 phosphate buffer, pH 5·3 to 7·7 for carbohydrates, M/10 citric acid—M/5 sodium phosphate buffer of McIlvaine, pH 3 to 6·6 followed by 0·2N phosphate- sodium hydroxide buffer of Britton and Welford, pH 6·6 to 12·2 for proteins, and the method adopted by Nicol (1930) for fat digestion. The enzyme-substrate mixtures were maintained at 33°C by electrically controlled thermostat.

The enzymes of the digestive diverticula.

From about 200 medium sized (12 cm. long) *Bankia indica* 7 gms. (wet weight) of digestive diverticula could be obtained and this would yield 1.33 gms., of a dry fawn coloured powder when treated with acetone. This acetone dried powder keeps well for a long time (Sumner and Somers, 1947) so that extracts could be made as and when required. The extracts of the diverticula appear to contain carbohydrases proteases, and lipases as the following experiments would show.

SECTION A

THE DIGESTIVE ACTION OF THE EXTRACTS ON CARBOHYDRATES

The action of the extracts on a wide range of carbohydrates like starch, sucrose, glycogen, cellobiose, maltose and lactose was tested (Experiment 1) using five ml. of Benedict's solution. Where reduction of dissacharides was expected Barfoed's test was also applied.

EXPERIMENT I.

TABLE I.

Action of the Enzyme on Carbohydrates

Temperature of the experiment 33°C.
pH of the experiment 6·2.
Control with boiled extract

2·5 ml. of the extract plus 10 ml. of the substrate plus 7·5 cc. of buffer solution.

Substrate	Duration of the experiment	Experiment Vol. required for titration in ml.	Control Vol. required for titration in ml.	Volume difference in ml.
4% starch solution	5 hrs.	1. 5·6	11·3	5·7
		2. 5·6	11·2	5·6
		3. 5·6	11·2	5·6
2% sucrose solution	5 hrs.	1. 6·5	13·8	7·3
		2. 6·5	13·9	7·4
		3. 6·6	14·0	7·4
1% glycogen	5 hrs.	1. 5·2	17·9	12·7
		2. 5·1	17·9	12·8
		3. 5·2	17·8	12·6
1% cellobiose	5 hrs.	1. 5·1	12·1	7·0
		2. 5·3	12·2	6·9
		3. 5·2	12·1	6·9
			1 ml. boiled with Barfoed's reagent.	
4% Maltose	48 hrs.	Reduction	No-reduction	
4% lactose	48 hrs.	Reduction	No-reduction	

The results obtained show that the digestive diverticula of *Bankia indica* contain an effectve carbohydrase enzyme system capable of digesting starch, sucrose, glycogen, maltose and lactose. The fact that it is able to simplify cellobiose, demonstrates the presence of cellobiase in the system. Since cellulose and pentosans are found in wood on which the teredines feed largely, the digestive action of the extract on saw dust filter paper, regenerated filter paper and gum-arabic was estimated. (Experiment 2). To check any changes due to autolysis, two controls, one with boiled extract and substrate and another with unboiled extract without substrate were maintained.

EXPERIMENT II.

TABLE II.

Action of the enzyme on cellulose and pentosans

Duration of the experiment—5 days.
Temperature of the experiment—33°C.
Initial pH of the experiment—6·2.

Digestive mixture	Experiment. Vol. required for titration in ml.		Control Vol. required for titration in ml.		Volume difference in ml.
	For 5 ml. of Benedict's solution				
			Control I	Control II	
2·5 ml. of the extract plus 1 gm. of saw dust in 10 ml. of distilled water plus 7·5 ml. of buffer solution.	1.	7·6	14·0	13·2	5·6
	2.	7·5	14·2	13·5	6·0
	3.	7·6	14·1	13·5	5·9
2·5 ml. of the extract plus 1 gm. of filter paper in 10 ml. of water plus 7·5 ml. of the buffer solution.	1.	13·7	14·9	13·8	0·1
	2.	13·6	15·1	13·9	0·3
	3.	13·7	15·0	13·7	0·0
2·5 ml. of the extract plus 0·5 gm. of regenerated filter paper in 10 ml. of water plus 7·5 ml. of the buffer solution. ..	1.	6·9	14·3	13·9	7·0
	2.	6·9	14·3	13·7	6·8
	3.	6·8	14·1	13·9	7·1
2·5 ml. of the extract plus 10 ml. of 5% gum arabic plus 7·5 ml. of the buffer solution. ..	1.	9·8	13·2	13·1	3·3
	2.	9·7	13·3	13·0	3·3
	3.	9·7	13·4	12·8	3·1

The results obtained for filter paper show that pure cellulose is not split into sugars by the enzyme extract. The results obtained for regenerated filter paper and saw dust establish that cellulase is present in the enzyme extract. It is also seen from the experiment that the enzyme can split pentosans as found in gum-arabic into assimilable sugars.

Summing up the results of the above two experiments one has to conclude that the extract of the digestive diverticula contains enzymes capable of decomposing several carbohydrates such as starch, sucrose, glycogen, cellobiose, maltose, lactose, saw dust, regenerated filter paper and gum-arabic many of which are present in some form or other in timber. It is noteworthy that cellulose which is not hydrolysed by the digestive enzymes of most animals can be acted on by two special enzymes namely cellulase and cellobiase present in the shipworm showing a close correlation between the enzyme equipment of the digestive diverticula and the substrate offered by the timber on which it largely feeds.

II

The optimum conditions for the activity of the enzyme.

Since all the carbohydrate splitting enzymes are likely to operate under more or less similar conditions, the optimum hydrogen-ion concentration, temperature, the influence of duration of digestion and the inter-dependence of these, were determined in relation to the digestion of starch alone.

(a) *The optimum hydrogen-ion concentration for the digestion of starch.*

The optimum hydrogen-ion concentration for maximal action of the enzyme on starch was determined as follows. (Experiment 3). Digestive mixtures were prepared, each consisting of 2·5 ml. of the extract, 10 ml. of 4% starch solution and 7·5 ml. of the various buffer solutions and toluol in ten test-tubes with different pH values ranging from 5·3 to 7·7. They were incubated at 33°C for 5 hours, boiled and made up to the original volume with distilled water. The incubation mixture was withdrawn from each tube and centrifuged to remove particulate material, the supernatant fluid was deproteinised and recentrifuged and 0·2 ml. aliquots were taken from the final supernatant and analysed for reducing sugars by the Somogy's method and compared with those obtained for the control tubes (vide Table III). The optimum

hydrogen-ion concentration for the digestion of starch was found to be between 6·2 to 6·5. The activity of the enzyme falls off rapidly on either side of this optimum and it is very little above pH 7·7 and below pH 5·3.

TABLE III.

The optimum pH for the digestion of starch.

2·5 ml. of the extract plus 10 ml. of 4% starch solution plus 7·5 ml. of buffer solution.

Temperature of the experiment—33°C.
Duration of the experiment—5 hours.

For control identical mixtures with boiled extract.

pH	Experiment Vol. in ml. of N/200 $Na_2S_2O_3$	Control Vol. in ml. of N/200 $Na_2S_2O_3$	Volume difference in ml.
5·3	14·62	15·9	1·28
5·6	13·86	15·88	2·02
5·9	12·66	15·92	3·26
6·2	10·46	15·80	5·34
6·5	10·62	15·88	5·26
6·8	13·98	15·96	1·98
7·0	14·06	15·88	1·82
7·2	14·48	15·9	1·42
7·4	14·48	15·68	1·20
7·7	14·98	15·9	0·92

Experiment 3 was repeated with a view to studying influence of the period of incubation on the optimum pH for maximal enzymic activity. Accordingly the incubation was continued for 10 hours and 16 hours with identical digestive mixtures. The results are presented in Table IV.

TABLE IV.

The relation between duration of digestion and optimum pH.

Temperature of the experiment—33°C.
Duration of the experiment—10 hrs., 16 hrs.

For control identical mixtures with boiled extract.

Duration of the experiment—10 hrs.

pH	Experiment Vol. in ml. of N/200 $Na_2S_2O_3$	Control Vol. in ml. of N/200 $Na_2S_2O_3$	Volume difference in ml.
5·3	14·5	15·68	1·18
5·6	13·96	15·72	1·76
5·9	13·62	15·8	2·18
6·2	9·52	15·72	6·2
6·5	9·56	15·74	6·18
6·8	13·58	15·70	2·12
7·0	13·93	15·84	1·91
7·2	14·05	15·60	1·55
7·4	14·48	15·62	1·44
7·7	14·66	15·74	1·08

Duration of the experiment—16 hours.

pH	Experiment Vol. in ml. of N/200 $Na_2S_2O_3$	Control Vol. in ml. of N/200 $Na_2S_2O_3$	Volume difference in ml.
5·3	13·08	14·9	1·82
5·6	12·62	15·02	2·4
5·9	12·34	15·04	2·7
6·2	8·08	14·98	6·9
6·5	8·38	14·98	6·6
6·8	12·64	15·08	2·44
7·0	13·02	15·02	2·00
7·2	13·22	15·04	1·82
7·4	13·55	15·0	1·45
7·7	13·76	15·02	1·26

Similarly to study the influence of temperature on optimum pH three identical sets were incubated at three different temperatures of 24, 33 and 42°C. (Experiment 4, Table V).

TABLE V

The influence of temperature on optimum pH.

2·5 ml. of the extract plus 10 ml. of 4% starch solution plus 7·5 ml. of buffer solution

Temperature of the experiment 24, 33, and 42°C.
Duration of the experiment—5 hours.
For control identical mixtures with boiled extract.

pH	Experiment Vol. in ml. of N/200 $Na_2S_2O_3$.	Control Vol. in ml. of N/200 $Na_2S_2O_3$.	Volume difference in ml.
Temperature 24°C.			
5·3	14·62	15·62	1·0
5·6	14·48	15·60	1·12
5·9	13·06	15·64	2·58
6·2	11·66	15·44	3·78
6·5	11·88	15·60	3·72
6·8	14·46	14·62	1·16
7·0	14·58	15·62	1·04
7·2	14·64	15·64	1·00
7·4	15·00	15·60	0·6
7·7	15·1	15·60	0·5
Temperature 33°C.			
5·3	14·42	15·9	1·48
5·6	13·86	15·88	2·02
5·9	12·66	15·92	3·26
6·2	10·46	15·80	5·34
6·5	10·62	15·88	5·26
6·8	13·98	15·96	1·98
7·0	14·06	15·88	1·82
7·2	14·48	15·9	1·42
7·4	14·78	15·68	0·9
7·7	14·98	15·9	0·92
Temperature 42°C.			
5·3	14·4	15·42	1·02
5·6	13·98	15·18	1·20
5·9	12·14	15·16	3·02
6·2	10·76	15·22	4·46
6·5	10·08	15·12	4·04
6·8	13·62	15·10	1·48
7·0	13·96	15·12	1·16
7·2	14·22	15·32	1·1
7·4	14·18	15·22	1·0
7·7	14·65	15·12	0·48

It was found that the optimum pH was not significantly altered either by a prolongation of the experiment or a change in the temperature of incubation and continued to be at about 6·2 to 6·5 suggesting a high degree of stability of the enzyme.

(b) *The optimum temperature for the digestion of starch.*

To determine the temperature at which maximum digestion is effected the following experiment was conducted (Experiment 5). Seven identical mixtures were incubated at the following temperatures: 25°C; 29°C; 33°C; 37°C; 42°C; 50°C and 70°C for four hours and the amount of reducing sugar formed after the experiment, was estimated. The optimum temperature for maximal enzyme action was found to be about 33°C (Table VI).

TABLE VI.

Determination of the optimum temperature for starch digestion.

Duration of the experiment 4 hrs.
pH of the experiment 6·2.
Control with boiled extract.

Digestive mixture.	Experiment Vol. in ml. of N/200 $Na_2S_2O_3$.	Control Vol. in ml. of N/200 $Na_2S_2O_3$.	Volume difference in ml.
2·5 ml. of the extract plus 10 ml. of 4% starch plus 7·5 ml of buffer at 25°C.	11·95	15·6	3·65
2·5 ml. of the extract plus 10 ml. of 4% starch plus 7·5 ml. of buffer at 29°C.	10·5	15·4	4·9
2·5 ml. of the extract plus 10 ml. of 4% starch plus 7·5 ml. of buffer at 33°C.	10·2	15·4	5·2
2·5 ml. of the extract plus 10 ml. of 4% starch plus 7·5 ml. of buffer at 37°C.	10·9	15·5	4·6
2·5 ml. of the extract plus 10 ml. of 4% starch plus 7·5 ml. of buffer at 42°C.	11·1	15·4	4·3
2·5 ml. of the extract plus 10 ml. of 4% starch plus 7·5 ml. of buffer at 50°C.	12·9	15·4	2·5
2·5 ml. of the extract plus 10 ml. of 4% starch plus 7·5 ml. of buffer at 70°C.	14·7	15·5	0·8

This optimum temperature for the enzyme action was not altered when the action of the enzyme was prolonged (Experiment 6) to 10, 15 and 20 hours at the optimum pH 6·2 (Table VII).

TABLE VII

The influence of time on temperature optimum

2·5 ml. of the extract and 10 ml. of 4% starch solution at various temperatures.
pH of the experiment—6·2
Control with boiled extract.
Duration of the experiment 5, 10, 15 and 20 hrs.

Temperature °C.	Experiment Vol. in ml. of N/200 $Na_2S_2O_3$.	Control Vol. in ml. of N/200 $Na_2S_2O_3$.	Volume difference in ml.
Duration of experiment 5 hours.			
24	11·8	15·7	3·9
29	10·6	15·6	5·0
33	10·1	15·5	5·4
37	10·7	15·6	4·9
43	11·3	15·7	4·4
47	12·5	15·9	3·4
52	12·9	15·7	2·8
Duration of experiment 10 hours.			
24	11·1	15·8	4·7
29	10·1	15·9	5·9
33	9·7	15·9	6·2
37	10·3	15·8	5·5
43	10·9	15·9	5·0
47	11·5	15·8	4·3
52	11·9	15·8	3·9
Duration of experiment 15 hours.			
24	10·3	15·9	5·6
29	9·4	15·8	6·4
33	9·1	15·8	6·7
37	9·9	15·9	6·0
43	10·3	15·8	5·5
47	10·9	15·7	4·8
52	11·6	15·8	4·2
Duration of experiment 20 hours.			
24	9·7	15·8	6·1
29	8·9	15·8	6·9
33	8·6	15·8	7·2
37	9·4	15·9	6·5
43	9·9	15·8	5·9
47	10·2	15·7	5·5
52	10·6	15·9	5·3

When identical sets of digestive mixtures were incubated at the various temperature as in the previous experiment at 3 pH levels, 6·2, 7·4 and 8·4 (Experiment 7, Table VIII). It was observed that the optimum temperature was always about 33°C.

TABLE VIII

Influence of pH on temperature optimum

2·5 ml. of the extract and 10 ml. of 4% starch solution at various temperatures.

Duration of the experiment—5 hrs.
Control with boiled extract.

pH of the experiment 6·2; 7·4; 8·4.

Temperature °C.	Experiment Vol. in ml. of N/200 $Na_2S_2O_3$.	Control Vol. in ml. of N/200 $Na_2S_2O_3$.	Volume difference in ml.
pH of the experiment 6·2.			
24	11·8	15·7	3·9
29	10·6	15·6	5·0
33	10·2	15·5	5·3
37	11·0	15·6	4·6
43	11·3	15·7	4·4
47	12·5	15·9	3·4
52	12·9	15·7	2·8
pH of the experiment 7·4.			
24	15·3	15·9	0·6
29	14·9	15·9	1·0
33	14·4	15·7	1·3
37	15·0	15·9	0·9
43	15·1	15·8	0·7
47	15·4	15·9	0·5
52	15·6	15·9	0·3
pH of the experiment 8·4.			
24	15·5	15·9	0·4
29	15·1	15·8	0·7
33	14·7	15·7	1·0
37	15·1	15·7	0·6
43	15·3	15·7	0·4
47	15·5	15·7	0·2
52	15·6	15·8	0·2

(c) *The temperature of destruction of amylase.*

Though from the point of view of understanding the digestion of carbohydrates by the action of the enzymes contained in the digestive diverticula, the temperature at which the enzymes cease to operate may not be of much biological significance, since *Bankia* is a tropical form where temperature ranges only from 26 to 31°C. yet in order to understand the thermal stability of the enzymes, principally amylase and cellulase, different samples of the extract were initially treated at various temperatures in a water-bath for fifteen minutes and then mixed with the substrate before incubation at the optimum temperature (33°C) as shown in experiment VIII, Table IX. It was found that while the optimum temperature was 33°C which is very near the temperature range of the habitat, the enzyme continued to be active even at 60°C and was almost completely inactivated at higher temperatures. It was therefore concluded that the enzyme is destroyed at about 60 to 65°C. The results are presented in Table IX.

TABLE IX

Determination of temperature of destruction of amylase

2·5 ml. of the extract heated for 15 minutes at various temperatures and 10 ml. of 4% starch solution plus 7·5 ml. of buffer solution.

Duration of the experiment 7 hrs.
pH of the experiment 6·2
Control with boiled extract.
Incubated at 33°C.
5 ml. aliquot samples for sugar estimation.

Temperature in °C.	Experiment Vol. in ml. of N/200 $Na_2S_2O_3$.	Control Vol. in ml. of N/200 $Na_2S_2O_3$.	Volume difference in ml.
40	11·1	15·9	4·8
45	11·3	15·9	4·6
50	12·9	15·9	3·0
55	13·2	15·9	2·7
60	15·92	15·9	1·98
65	15·75	15·9	0·15
70	15·75	15·9	0·15
80	15·7	15·9	0·2
90	15·8	15·9	0·1
98	15·8	15·9	0·1

The experiment VIII was repeated with saw dust as the substrate and the results presented in Table X show that cellulase is destroyed at a temperature of 70°C.

TABLE X

2·5 ml. of the extract heated for 15 minutes at various temperatures and 1 gm. of sawdust in 10 ml. of distilled water plus 7·5 ml. of buffer solution.

Duration of experiment — 5 days.
Initial pH of the experiment 6·2.
Control with boiled extract.
Incubated at 33°C.

Temperature in °C.	Experiment Vol. in ml. of N/200 $Na_2S_2O_3$.	Control Vol. in ml. of N/200 $Na_2S_2O_3$.	Volume difference in ml.
40	14·35	16·5	2·15
45	14·42	16·5	2·08
50	14·48	16·5	2·02
55	14·5	16·5	2·0
60	14·58	16·5	1·92
65	14·70	16·5	1·8
70	15·48	16·5	1·02
80	15·58	16·5	0·92
90	15·58	16·5	0·92
98	15·58	16·5	0·92

(d) *Effect of variation of concentration of enzyme* (*Experiment XII*).

0·5 gm. of the acetone dry powder was extracted with 72 ml. of distilled water and the following volumes of extract were added, 16 ml., 14 ml., 12 ml., 10 ml., 8 ml.; and 4 ml. The substrate consisted of 10 ml. of starch and 10 ml. of buffer solution (pH 6·2). The total volume was made up in each case to 36 cc. The concentrations at the different levels are indicated as 1, 7/8, 3/4, 5/8, 1/2, 3/8 and 1/4. The results are presented in Table XI. It was observed that the velocity of reaction was not in direct linear proportion to the quantity of enzyme present at the levels studied. Higher concentrations of the enzyme were comparatively less active than lower ones as could be observed, from the table (the maximal enzyme concentration I gave a volume difference of 8·78 while the concentration 3/8 gave a value of 5·36).

TABLE XI

Influence of variation of concentration of the enzyme

Duration of the experiment — 3 hrs.
Temperature of the experiment — 33°C.
pH of the experiment — 6·2.
Control with boiled extract.

Vol. of extract in 16 ml. of solution.	Substrate.	Enzyme concentration in 36 ml. of digestive mixture.	Vol. in ml. of N/200 $Na_2S_2O_3$ experiment.	Vol. in ml. of N/200 $Na_2S_2O_3$ water blank.	Volume difference in ml.
16	10 ml. of 1% starch + 10 ml. of buffer.	1	11·42	20·2	8·78
14	2 ml. of water + 10 ml. of 1% starch + 10 ml. of buffer.	7/8	11·76	20·2	8·44
12	4 „ „ „	3/4	12·56	20·2	7·64
10	6 „ „ „	5/8	13·16	20·2	7·04
8	8 „ „ „	1/2	13·92	20·2	6·28
6	10 „ „ „	3/8	14·84	20·2	5·36
4	12 „ „ „	1/4	15·55	20·2	4·65

TABLE XII

Influence of variations of concentration of the substrate

Duration of the experiment — 3 hours.
Temperature of the experiment — 33°C.
pH of the experiment — 6·2.
Control with boiled extract.

Enzyme in ml.	Substrate.	Concentration of starch in 30 ml. D. mixture.	Vol. in ml. of N/200 $Na_2S_2O_3$ required for titration.		
			Experiment.	Water blank.	Vol. difference in ml.
10	10 ml. of 1% starch + 10 ml. of buffer.	1	10·8	20·2	9·4
10	10 „ of 0·75 % „	3/4	12·3	20·2	7·9
10	10 „ of 0·5 % „	1/2	13·0	20·2	7·2
10	10 „ of 0·25 % „	1/4	15·8	20·2	4·4
10	10 „ of 0·125 % „	1/8	16·0	20·2	4·2
10	10 „ of 0·0625 % „	1/16	16·7	20·2	3·5
10	10 „ of 0·03125% „	1/32	18·0	20·2	2·2

Effect of variation of concentration of substrate (Experiment XII).

In order to determine the influence of the variation of the concentration of the substrate upon the action of the enzyme 0·5 gm. of acetone dry powder was extracted with 70 cc. of distilled water with the following levels of substrate concentration: 1, 3/4, 1/2, 1/4, 1/8, 1/16 and 1/32 as shown in table XIII, the total volume of the mixture in each case being 30 ml.

It will be seen that as the substrate concentration is increased the degree of enzyme action also increases. At starch concentration below 1/8 the activity seems to be a function of the substrate concentration and at concentration 1/8 to 1/4 the substrate concentration had no significant influence on enzyme activity i.e. the reaction is of zero order. At concentration of the substrate higher than 1/4 the activity with again proportional to the concentration of the substrate.

SECTION B

THE DIGESTION OF PROTEINS

The study of enzymic proteolysis has not received sufficient attention from earlier workers who studied the digestion in the wood-boring teredines. This is probably because wood is known to have a very low protein content (Miller, 1923), and consequently it was assumed that the proteolysis in the wood-boring molluscs was comparatively unimportant. It has recently been shown that the wood is qualitatively inadequate as a source of nitrogen for *Teredo* (Lasker and Lane, 1953) and that the latter satisfied its nitrogen requirements by making use of the proteins both from the wood and from particulate matter suspended in water. In view of this fact that wood-boring molluscs can consume the protein-rich nannoplankton of sea water, it was considered worthwhile investigating the proteolytic enzyme systems contained in the digestive diverticula of *Bankia indica*. Typical proteins like gelatine, fibrin and casein as well as commercial peptone were supplied as substrate to study the enzymic activity of the extracts of the digestive diverticula. The free amino-acids liberated were qualitatively detected by bromine water and the amino-nitrogen formed were estimated by Srensen's formol titration. The results are presented in the table XIII. The protease of the enzyme extract was found to have no effect on coagulated albumen.

TABLE XIII

Action of the enzyme extract on proteins

Temperature of the experiment 33°C.
Duration of the experiment 48 hours.
Initial pH of the experiment 7.
Control with boiled extract.

Digestive mixture.	Experiment Vol. of N/20 in ml. required for titration.	Control Vol. of N/20 NaOH in ml. required for titration.	Volume difference in ml.
2·5 ml. of the extract plus 5 cc. of 2% gelatine plus 5 ml. of buffer solution.	1. 4·06 2. 4·12	3·08 3·36	0·98 0·76
2·5 ml. of the extract plus 5 gm. of fibrin in 5 ml. of water plus 5 ml. of buffer solution.	1. 3·64 2. 3·58	3·28 3·28	0·36 0·30
2·5 ml. of the extract plus 5 ml. of 2% peptone plus 5 ml. of buffer.	1. 6·04 2. 6·10	4·88 4·80	1·16 1·30
2·5 ml. of the extract plus 5 ml. of 2% casein plus 5 ml. of the buffer.	1. 3·88 2. 3·82	3·52 3·50	0·36 0·32

It will be evident from the table that the extracts of the digestive diverticula have active proteolytic enzymes and that peptidase activity is more marked than that of protease. In general both the protease and peptidase activities are poor compared to those of the extracts of other lamellibranchs. It is of interest to point out the close correlation between the weak nature of the protease and the low nitrogen content of the adult shipworm (Greenfield, 1953).

The optimum pH for maximal activity for protease and the thermal destruction point of the enzyme acting on gelatine substrate were studied.

Optimum hydrogen-ion concentration for the digestion of proteins.

Experiment (Expt. 15) on the digestion of gelatine over a pH range of 3 to 12 showed (Table XIV) that there are two optimal

pH values namely 4.6, and 8.5. It is probable that this double optima might be due to the presence of two separate protolytic enzymes, one active in an acid medium and the other in an alkaline medium.

TABLE XIV.

Influence of Hydrogen-ion concentration on the digestion of gelatine

2·5 cc. of the enzyme extract plus 5 cc. of 2% gelatine plus 10 cc. of buffer mixture.

Control. 2·5 cc. of the enzyme extract (boiled) plus 5 cc. of 2% gelatine plus 10 cc. of buffer mixture.

Temperature of the experiment—33°C.

Duration of the experiment—24 hours.

Buffer mixture.

McIlvaine pH 3-6·6.

Britton and Welford pH 6·6-12·02.

Average pH.	Experiment Vol. of N/20 NaOH in ml. required for titration.	Control Vol. of N/20 NaOH in ml. required for titration.	Volume difference in ml.
3	2·35	2·34	0·01
3·6	2·42	2·30	0·12
4·0	2·76	2·62	0·14
4·6	4·29	2·44	1·85
5·0	4·02	2·30	1·72
5·6	2·98	2·02	0·96
6·0	3·56	2·94	0·62
6·6	3·46	3·04	0·42
7·0	3·50	2·88	0·62
7·75	4·62	2·28	2·34
8·5	4·98	2·26	2·72
9·2	3·84	2·24	1·6
10·8	2·71	2·20	0·51
11·5	1·42	1·20	0·22
12·02	1·2	1·00	0·20

Temperature of destruction of protease: (*Expt.* 16).

The temperature of destruction of protease was determined by heating the extract to various temperatures from 40 to 98°C for 15 minutes and subsequently incubating with a 2% solution of gelatine at 33°C. The results are presented in Table XV.

TABLE XV

Temperature of Destruction of Protease

2·5 ml. of the extract heated for 15 minutes at various temperatures plus 5 ml. of 2% gelatine plus 10 cc. of the buffer solution.

Duration of the experiment — 48 hours.
pH of the experiment — 7.
Incubated at 33°C.

Temperature °C.	Vol. of N/20 NaOH in ml. required for titration.
40	.. 3·75
45	.. 3·62
50	.. 2·94
55	.. 2·15
60	.. 1·52
65	.. 1·10
70	.. 0·80
75	.. 0·80
80	.. 0·80
90	.. 0·80
98	.. 0·80

The temperature of destruction of protease after a 15 minute's heating at pH 7 lies between 65 and 70°C.

SECTION C

The Digestion of fats.

Since Red-cedar like other cedars contains oily constituents the lipolytic capacity of the enzyme extracts was studied.

To test the presence of a lipase in the digestive diverticula of *Bankia indica* 5 ml. of the extract was added to 5 ml. of boiled

milk which was made alkaline to phenol red by adding sufficient quantity of N/200 NaOH solution. A control was maintained with boiled extract. Both the mixtures were incubated at 33°C. The control showed no change even after 24 hours, whereas the colour in the experimental tube turned yellow suggesting that the lipase present in the extract converted butyrin into butyric acid (Yonge 1924).

The lipolytic enzyme was found (Experiment 17, Table XVI) capable of hydrolising typical fats such as methyl acetate, lecithin solution and olive oil emulsion. The increase in acidity due to the liberation of fatty acid by the hydrolytic action of lipase was recorded in terms of N/100 NaOH.

TABLE XVI

Action of Enzyme Extracts on Fats

Temperature of the experiment — 33°C.
Duration of the experiment — 48 hrs.
pH of the experiment — 7·3.
Control with boiled extract.

Digestive mixture.	Experiment Vol. in ml. of N/100 NaOH required for titration.	Control Vol. in ml. of N/100 NaOH required for titration.	Vol. difference n ml.
2·5 ml. of the extract + 2·5	11·5	8·85	2·65
ml. of 2% Methyl acetate + 5 ml. of the buffer solution.	11·5	3·9	2·6
2·5 ml. of the extract + 2·5	3·12	2·32	0·8
ml. of 2% lecithin solution + 5 ml. of the buffer solution.	3·2	2·34	0·86
2·5 ml. of the extract + 2·5	5·7	4·62	1·08
ml. of olive oil + 5 ml. of the buffer solution.	5·9	4·6	1·30

The optimum hydrogen-ion concentration for the digestion of fat was found to lie at about pH 7·3 (Experiment 18, Table XVII). The lipolytic activity decreased above pH 7·3 and practically ceased below pH 5·8.

TABLE XVII

The influence of the digestion of methyl acetate

2·5 ml. of the extract and 2·5 ml. of the 2% methyl acetate solution plus 5 ml. of the buffer solution.

Duration of the experiment — 48 hrs.
Temperature of the experiment — 33°C
Control with boiled extract.
Buffer — Northrop's modified.

Average pH.	Experiment Vol. of N/100 NaOH in ml. required for titration.	Control vol. of N/100 NaOH in ml. required for titration.	Volume difference in ml.
4·6	17·9	18·1	0·2
5·8	16·6	16·2	0·4
6·5	14·7	14·0	0·7
6·9	12·4	11·2	1·2
7·3	11·8	9·3	2·5
7·6	11·0	9·0	2·0
7·8	10·1	8·6	1·5
8·2	9·3	8·2	1·1
8·5	8·7	7·9	0·8
8·9	7·5	7·1	0·4

The temperature of destruction of the lipase was found as in the previous experiments by heating the extract at different temperatures for fifteen minutes in a water bath and then incubating with methyl acetate solution (Experiment 19, Table XVIII).

The hydrogen-ion concentration of the gut contents.

The hydrogen-ion concentration of the guts from six specimens of *Bankia indica* was determined by the spot method of Clark and Lubs. Fresh specimens were dissected and portions of the gut contents after mixing with a drop of distilled water was tested with the indicator. It was observed from the readings that the stomach in all cases was the most acid region of the gut the average pH being 5·8. This acidity is caused by the dissolution of the crystalline style which has a pH of 5·5. In the caecum the pH increases, the average being 6·1. In the digestive diverticula the pH was found to be 6·3 and in the midgut it rises to 7·3 while the rectum showed an average pH of 7·6. The average pH of the oesophagus was 6·4 and that for the mantle cavity was 7·9.

The optimum hydrogen-ion concentration for the action of the enzymes of both the digestive diverticula and the crystalline style is almost the same as detected in those parts of the alimentary canal of the animal where the enzymes are normally effective. However, in the case of the protease it falls beyond the limits of the average pH of the gut. Similar conditions have been reported by earlier authors such as Yonge (1926) for the protease of the Oyster, and Nicol (1930) for part of the range of amylase and protease in *Sabella pavonina*.

Discussion

It is well known that most animals are incapable of digesting cellulose because they lack the enzyme equipment necessary for the hydrolysis of this highly resistant substance which forms the bulk of the dry weight of wood. That the ship-worms which live chiefly on wood are an exception is evident because of the strong carbohydrase system of enzymes present in the crystalline style and digestive diverticula. The capacity to elaborate cellulose, cellobiase and enzymes capable of hydrolysing pentosans in addition to the other carbohydrase enzymes is as much an adaptation to their peculiar diet as the modification of alimentary canal, the shell, or the foot. This fact is obvious when we find that the capacity which other lamellibranchs, such as *Pecten, Mya* and *Ostrea* possess in splitting not only carbohydrates but proteins and fats as well is retained by *Bankia indica*. Closely correlated with this emphasis on the digestion of carbohydrates is the capacity for the storage of great quantities of glycogen (Lane, Posner and Greenfield, 1952; Greenfield, 1953).

The conclusions of Johnson *et al.* (1936) and Bartsch (1922) that the ship-worm can live on plankton alone even after all the available wood is exhausted appear to contradict the finding of Potts (1923) and Roch (1947) that the supply of wood is the principal factor affecting the life of this mollusc. Since these wood boring molluscs are equipped with all the three sets of digestive enzymes, these must obviously be capable of living not only on wood but also on other items of food both living and non-living, brought into the body along with the respiratory current of water. Conclusive tests based on the exclusion of all water borne material are probably difficult to conceive. Even under normal conditions *Bankia* may not be living on wood alone but on plankton as well as recently shown by Lakser and Lane (1953) for *Teredo.* However, it is not improbable that other factors such as the high specialisation in the sorting mechanisms which permit only an insufficient quantity of the finest particles to reach the site of digestion from the respiratory current, the major part being rejected as 'pseudofaeces' might be one of the causes of their reported mortality after the wood supply is exhausted. Further, there is also the factor of over-crowding which turns the timber into a porous, highly fragile structure which would permit the entry or parasitic protozoan and harmful bacteria—a serious menace to the life of the community as found by Grave (1928). Probably it is under these conditions that shipworms die and not because of dire starvation.

It will be seen that the simplification of the cellulose into assimilable sugars is accomplished by enzymes both from the crystalline style and the digestive diverticula. The role of the crystalline style seems to be a preparatory one acting on large molecules reducing the food into simpler soluble state enabling considerable extracellular digestion of the non-nitrogenous food to take place in the stomach and the caecum, especially of the large wood fibres which cannot be taken whole into the cells of the specialised region of the digestive diverticula (Nair, 1955a, 1956, 1957). It is evident that the style splits the cellulose into the intermediary cellobiose and probably passing it on for the intracellular digestion in the diverticula (Nair, 1956). It is not known whether this disaccharide cellobiose can be directly absorbed by the coiled typhlosole of the caecum. Thus the cellulose splitting seems to take place in steps in two different sites, one extra cellularly in the caecum and the other intracellularly in the vacuoles of the digestive diverticula which has a cellobiase powerful enough to complete the diges-

tion before absorption, thereby exploiting to the fullest measure the nutrient resources of the wood. Phagocytes playing an important role in the digestive process is not, however, improbable for Yonge (1926, 1926a) has noticed phagocytes around the stomach, digestive diverticula, and midgut passing into the lumen of the gut ingesting particles of food which they later carry back to the tissues and digest.

For a proper understanding of the digestive enzymes of *Bankia,* the various properties of the enzymes and the conditions under which they operate deserve reference. In the foregoing study an attempt has been made to find out the influence of the hydrogen-ion concentration and temperature on enzyme action. It has been shown in several animals that the action of the digestive enzyme is dependent on the hydrogen-ion concentration of the substrate (Roaf, 1906; Yonge 1926; 1931; 1937; Vonk 1937). In *Bankia indica* the digestive activity of the amylase of the diverticula is at its maximum in an acid medium (6·2 to 6·5) as in *Mya* (Yonge, 1923) and *Ostrea* (Yonge, 1926). It was observed that changes in the duration of the experiment and temperature do not affect the pH optimum. Graham (1931b) has shown that in *Pecten maximus* the pH optimum is not affected by variations in time of the experiment. The optimum temperature for the amylase of *Bankia indica* also is unaltered by changes in the duration of the experiment and the pH of the substrate as was observed in *Sabella* (Nicol, 1930). However, Berril (1929), found in the ascidian *Tethyum* a lowering of temperature optimum when duration of the experiment is prolonged. Graham (1931b) showed in *Pecten* the existence of a direct relationship between the pH and optimum temperature of enzyme action. The results obtained for *Bankia* are suggestive of the relative stability of the amylolytic enzyme. The pH temperature and other such conditions influencing the operation of the enzyme may differ in different lamellibranchs as well as in the same animal itself, the enzymes of the style differing from those of the digestive diverticula. Other findings such as the optima of protease not being available in any part of the gut, the optimum temperature of amylase which is slightly above that of the animal and its environment and facts like these are probably due to the enzymes being tested *in vitro.*

In assessing the values of these results of the digestive enzymes of the living animal, it must be admitted that the substrats used to detect the range in these laboratory experiments and the

hydrolytic activity of the enzyme on these comparatively pure substances throw light on the general properties and specificity of the enzymes without reference to the actual range of operation in a state of nature in the organism-environmental set up. Supply of the different natural foods as substrates to assess the range of activity is not practicable since we have no information regarding the exact composition of the food of *Bankia* much less about the chemistry of these food stuffs.

Similarly, Yonge (1937) has questioned whether experiments conducted with large quantities of extracts of the digestive tissues, have any significant bearing on the condition in the ciliary feeding animals where the enzyme present in the lumen at any time is so much less than the amount used in the experiments, and where the time of passage through the gut bears little relation to the effective duration of enzyme action because the great part of the time is spent in the non-absorbing intestine and rectum.

This very true observation has been borne in mind throughout the tests, the results of which are offered in this paper, the proportions between the enzyme extract and the substrate, as well as the duration of the tests have been as far as possible adjusted to reproduce conditions found in this wood-borer where unlike other lamellibranchs the food is retained in the caecum and digestive diverticula for a long period.

The fat-splitting enzymes of invertebrates have been shown to be different in their composition from those of vertebrates (Vonk, 1937). Though the range of the lipolytic enzymes of *Bankia* is apparently wide, as may be inferred from its digestive action on lecithin, olive oil, and methyl acetate, it is comparatively weak. Similar activity has been noticed in other lamellibranchs (Dakin 1909; Yonge 1923; 1926).

It has been shown (Yonge 1923; 1926; 1937; Berril, 1929; Graham 1931a) that in lamellibranchs, herbivorous gastropods, and tunicates, the proteolytic enzymes are very weak. The protease present in the extracts of the digestive diverticula is similarly weak but its range of action is wide, capable of simplifying typical proteins such as fibrin, casein, gelatine and peptone. The protease was found to have two optima, one at pH 4·6 and the other at pH 8·5 when gelatine was the substrate. Yonge (1926) observed that the protease had two optima at pH 3·7 and pH 9·0, for *Ostrea* and Graham (1931a) found a similar double optima (pH 4·2 and 8·2)

for *Ensis siliqua*. It is possible that the two optima observed may be due to the presence of two separate enzymes as Bodansky and Rose (1922) believed, one from the digestive diverticula and the other from the phagocytes (Yonge, 1926). Krukenberg (1878) made use of the reasoning that a protease working in the acid medium is pepsin, while that acting in alkaline medium must be trypsin. However later workers (See Yonge, 1926) have rejected such uncritical definitions from vertebrate physiology. The low protease activity of the digestive diverticula is in conformity with the findings of Greenfield (1953) that the nitrogen content of the adult *Teredo* is generally low.

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Carbon Nitrogen Metabolism of Soil Fungi—III

BY

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ABSTRACT

Glucose-ammonia nitrate media behave like a true nitrate media in respect that pH tends towards neutrality. Fusaria appear to utilise nitrate nitrogen preferentially since ammonia nitrogen concentration remain practically constant for any particular level of glucose. This may be only an apparent phenomenon, the rate of production of ammonia nitrogen from the nitrate is, in the steady state, the same as its utilisation by the fungus. The rate of glucose depletion from the medium and nitrogen accumulated in the mat follow an exponential law. The rate of growth varies with the concentration of glucose with an optimum C/N ratio for maximum growth.

In the previous parts the author (Natarajan) had noted that the pH of the culture medium after an initial lowering with sucrose as the carbon source, rose towards neutrality when sodium and potassium nitrates formed the nitrogen sources. However when ammonium sulphate and ammonium chloride formed the nitrogen sources, the pH showed a clear tendency towards greater acidity; but sucrose-ammonium nitrate medium behaves like other nitrate media in that hydrogen-ion concentration after an initial lowering rose towards neutrality. The difference noticed using ammonium nitrate is presumably because the ammonia nitrogen concentration remains apparently steady for any particular level of sucrose and hence Fusaria are represented to utilise nitrate nitrogen preferentially. The rate of growth varies with the concentration of sucrose with an optimum C/N ratio for maximum growth. Both Fusaria displayed different physiological relationship. This is evident from their rates of growth, their nitrogen and sucrose utilisation and the associated changes of the media caused by them. These variations suggested that it is desirable to study the variation in glucose utilisation when the nitrogen source is ammonium nitrate.

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Materials and Methods

The culture methods and experimental technique that were employed in this work were as described earlier (Natarajan). The two fungi *Fusarium vasinfectum* and *Fusarium udum* were grown in 50 ml. of Richards synthetic liquid medium (Rawlins 1933) in 250 ml. Hysil Erlenmeyer flasks. Four levels of glucose were tried with ammonium nitrate as the nitrogen source. Besides nutrient salts 50 ml. of the medium contained:—

Level I. 500 mg. of Glucose + 70 mg. of Nitrogen in the form of ammonium nitrate;

Level II. 1000 mg. of Glucose + 70 mg. of Nitrogen in the form of ammonium nitrate;

Level III. 2500 mg. of Glucose + 70 mg. of Nitrogen in the form of ammonium nitrate;

Level IV. 5000 mg. of Glucose + 70 mg. of Nitrogen in the form of ammonium nitrate.

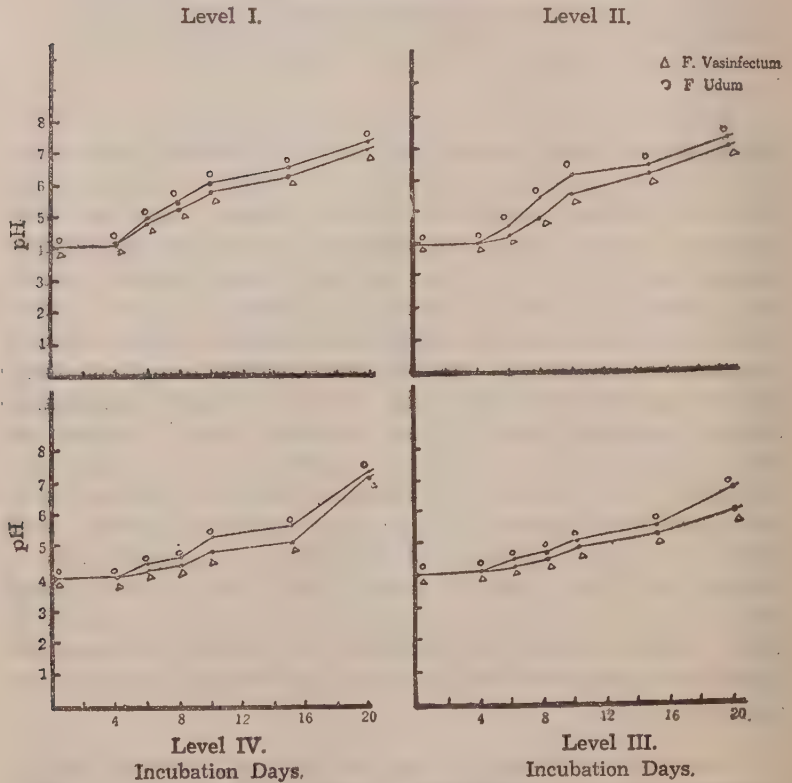

FIG. 1. pH changes of the fungi in different concentrations of media.

Data and Discussion

With glucose ammonium nitrate media, the initial pH 4 is maintained till the 4th day at all concentrations of glucose, thus differing from sucrose nitrate combination (Fig. 1). At pH 4, nitrate ions can function as oxidising agents and there may be an initial lowering in pH in glucose nitrate media also. By the fourth day, pH begins to rise and reaches the original value. Some ammonia nitrogen has also been utilised initially as in the presence of sucrose but, at the same time more nitrate nitrogen is found to be utilised than in sucrose nitrate media (Table 1). Also apparently the nitrate nitrogen utilised is greater in amount than the corresponding ammonia nitrogen, thus accounting for the

TABLE 1.

Residual Nitrogen (mg).

Sucrose series.

Days of incubation.	*F. vasinfectum*			*F. udum*	
	Nitrate nitrogen (mg)	Ammonia nitrogen (mg)		Nitrate nitrogen (mg)	Ammonia nitrogen (mg)
4	34·81	31·25	Level I	34·85	31·15
6	34·64	31·20		34·57	31·08
4	34·85	31·19	Level II	34·85	31·15
6	34·45	31·12		34·81	31·12
4	34·84	28·32	Level III	34·82	25·27
6	22·70	28·30		29·15	25·20
4	34·86	28·06	Level IV	34·85	27·50
6	32·74	28·00		34·24	26·98
			Glucose series.		
4	31·01	34·50	Level I	31·21	34·50
6	29·91	34·20		30·11	34·20
4	29·10	34·12	Level II	28·91	34·12
6	22·80	34·11		26·41	34·10
4	28·80	34·10	Level III	31·95	34·13
6	21·60	34·12		28·05	34·12
4	31·00	34·10	Level IV	29·80	34·20
6	29·60	34·10		28·10	34·20

TABLE 2.

(Nitrate Nitrogen = Ammonia Nitrogen = 35 mg. initially) Residual Nitrogen (mg.) Glucose Series.

Days of incubation.	LEVEL I.		LEVEL II.		LEVEL III.		LEVEL IV.	
	Nitrate Nitrogen.	Ammonia Nitrogen.	Nitrate Nitrogen	Ammonia Nitrogen.	Nitrate Nitrogen.	Ammonia Nitrogen.	Nitrate Nitrogen	Ammonia Nitrogen.
F. Vasinfectum.								
4	31·01	34·50	29·10	34·12	28·80	34·10	31·00	34·10
6	29·91	34·20	22·80	34·11	21·60	34·12	29·60	34·10
8	29·00	34·20	21·40	34·10	18·00	34·11	26·00	34·10
10	26·75	34·20	18·30	34·10	15·10	34·11	20·40	34·10
15	26·47	34·19	15·70	34·10	10·50	34·11	19·20	34·08
20	26·33	34·19	15·00	34·10	3·40	34·11	18·10	34·08
F. Udum.								
4	31·21	34·50	28·91	34·12	31·95	34·13	29·80	34·20
6	30·11	34·20	26·41	34·10	28·05	34·12	28·10	34·20
8	29·21	34·11	26·00	34·10	18·05	34·12	27·98	34·20
10	27·56	34·10	23·00	34·10	15·75	34·12	24·98	34·20
15	26·90	34·10	22·78	34·10	11·85	34·12	24·53	34·20
20	26·55	34·10	22·65	34·10	7·65	34·12	24·23	34·20

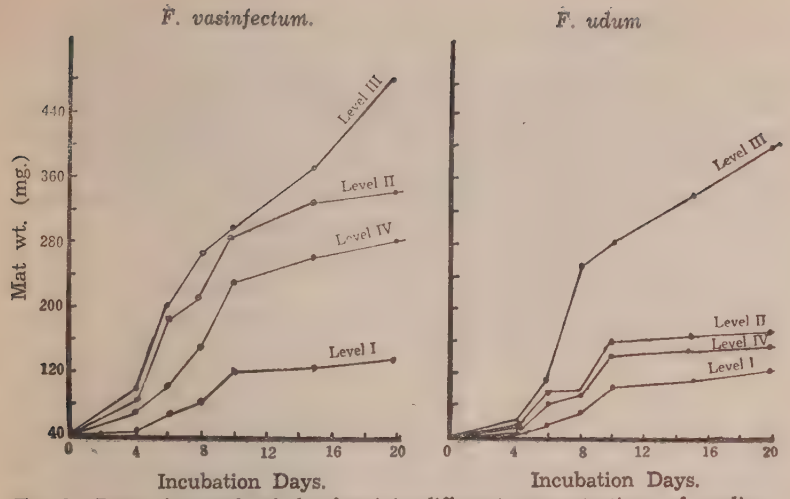

FIG. 2. Rate of growth of the fungi in different concentrations of media.

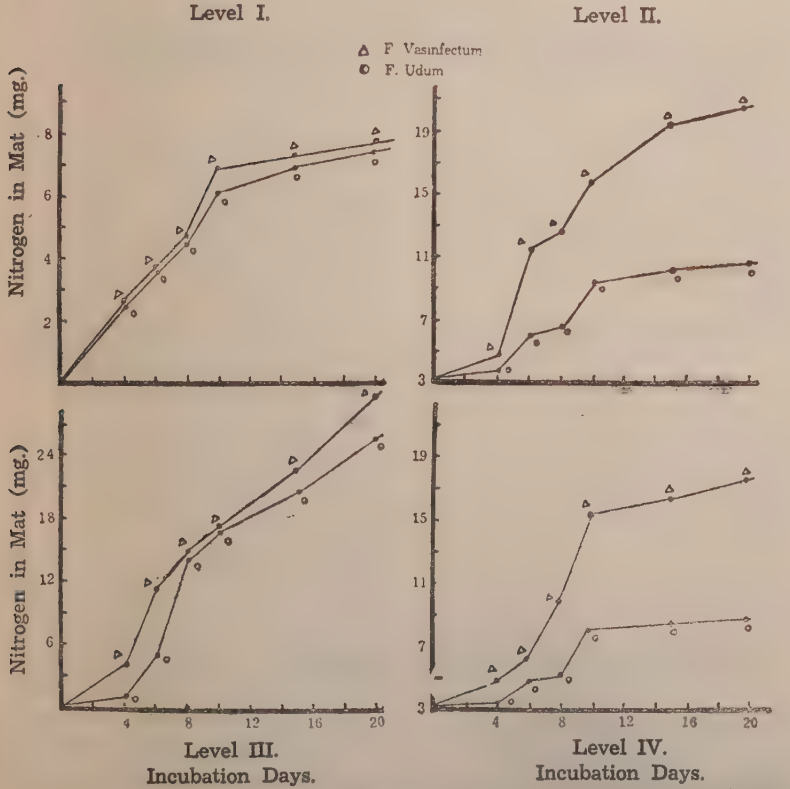

FIG. 3. Rate of nitrogen accumulation in the mat of the fungi at different concentrations of media.

absence of an initial lowering of pH. Another interesting observation is that more of nitrate nitrogen and less of ammonia nitrogen are utilised with glucose as the carbon source rather than Sucrose, at all levels with both fungi (c.f. Table I of part II, Natarajan). With both carbon sources, after the initial utilization of ammonia nitrogen, ammonia nitrogen remains apparently constant for any particular level and nitrate nitrogen alone appears to be utilised preferentially by both Fusaria (Table 2). This is easily accounted for if the rate of production of ammonia nitrogen from the nitrate, in the steady state, is the same as its utilisation by the fungus.

F. udum changes the pH more rapidly than *F. vasinfectum* after the fourth day, at all levels of glucose towards neutrality (Fig. 1). Even though the pH changes are more rapid than with *F. udum* the growth is more pronounced in the case of

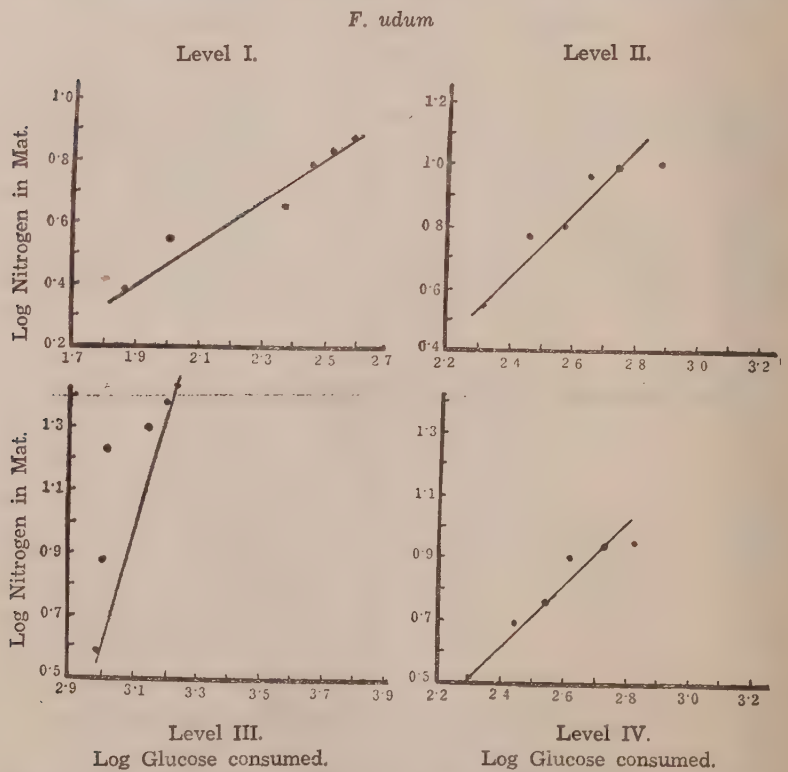

FIG. 4. Logarithmic relationship in *F. udum* at different concentrations of media.

F. vasinfectum as the mat weight is greater at all levels (Fig. 2) and more of nitrogen is taken up by the mat of *F. vasinfectum* at all levels of glucose (Fig. 3). The rate of glucose depletion from the medium and nitrogen accumulated in the mat follow an exponential law (Fig. 4, 5). Mat weight is the maximum with

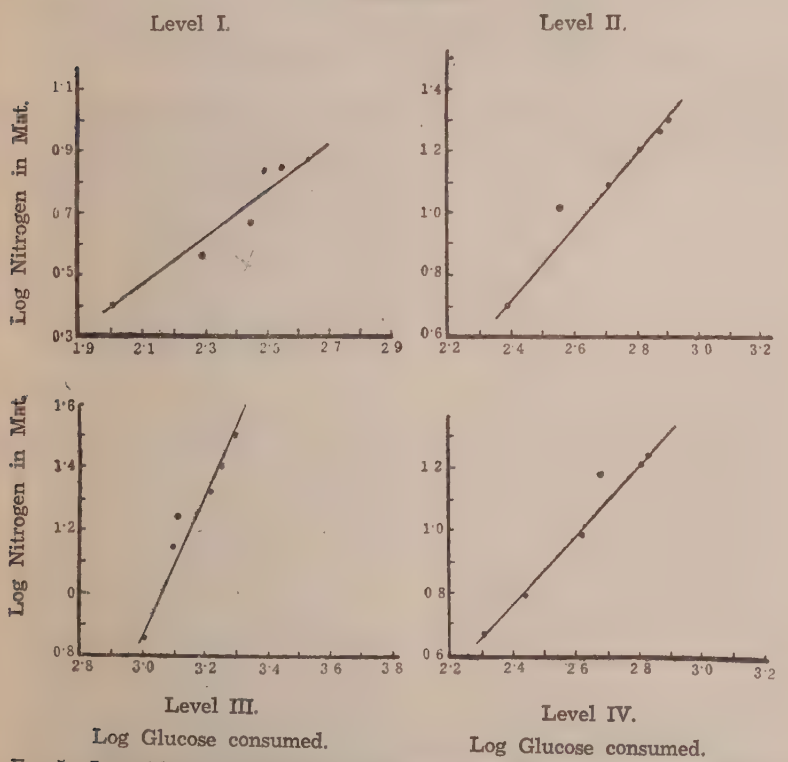

FIG. 5. Logarithmic relationship in *F. vasinfectum* at different concentrations of media.

third level the order being III > II > IV > I in the case of glucose (Fig. 2) and III > IV > II > I in the case of sucrose with both fungi (c.f. Fig. 2 of part II Natarajan). In every case (with both fungi) at all levels investigated, the total residual nitrogen differs from the sum of ammonia nitrogen and nitrate nitrogen. This difference increases with increasing periods of incubation and depends on nitrogen as well as the carbon source (c.f. Table IV of part II). At the third level this difference is found to be a minimum. On the 4th day it is equal to zero.

Insufficient glucose as well as excess of glucose reduce fungal growth. At level III which is the optimum level for growth, this difference is a minimum (Table 3). Steinberg (1939 a) found the

TABLE 3.

Difference (mg), i.e., Residual total nitrogen — (residual nitrate nitrogen + ammonia nitrogen).

Days of incubation.	Level I	Level II	Level III	Level IV
	F. vasinfectum			
0 ..	0·0	0·0	0·0	0·0
4 ..	1·92	1·88	0·0	0·01
6 ..	2·26	1·98	0·02	0·09
8 ..	2·28	2·00	0·04	0·14
10 ..	2·29	2·02	0·13	0·17
15 ..	2·31	2·12	0·19	0·28
20 ..	2·32	2·18	0·20	0·33
	F. udum			
0 ..	0·0	0·0	0·0	0·0
4 ..	1·88	3·56	0·07	2·69
6 ..	2·18	3·65	0·13	2·77
8 ..	2·32	3·65	0·19	2·77
10 ..	2·36	3·68	0·18	2·86
15 ..	2·37	3·69	0·22	2·86
20 ..	2·37	3·70	0·23	2·88

mat weight increases with the increase in the concentration of sugar for the same concentration of NH_4NO_3 with an optimum carbohydrate concentration. He found that there is a definite C/N ratio changes in the medium fat synthesis falls off markedly, presumably because the carbohydrate is diverted to protein synthesis. For the Fusaria investigated with increasing concentration of carbohydrate (glucose) keeping the Nitrogen concentration same, it is found that dried mycelial weight increases till third level (glucose) and then decreases. Any fungus thus needs specific

nitrogen concentrations for any one carbohydrate level for maximum activity.

The ability to utilise nitrate nitrogen as well as ammonia nitrogen represents a greater versatality on the part of a fungus than one which uses ammonia nitrogen alone (Foster 1949). The present investigations along with other work from these laboratories reveal that Fusaria can use nitrate nitrogen and also ammonia nitrogen irrespective of carbon sources (glucose or sucrose).

Bacteria exhibit the following phases of growth:—

1. Stationery phase.
2. Phase of accelerated growth.
3. Exponential phase.
4. Phase of declining acceleration.
5. Maximum stationery phase.
6. Phase of decline.

Fungi in general follow the same order of the phases of growth but the exponential phase of growth is often considered to be absent. However, in the present study, with *F. vasinfectum* and *F. udum,* when the logarithm of carbohydrate utilisation of the fungus are plotted against logarithms of nitrogen accumulation of the mat, a linear relationship is obtained. This suggests that both the rate of carbohydrate depletion and that of nitrogen accumulation on the mat follow an exponential law. The apparent absence of an exponential phase may be attributed to the more complex nature of the organism. This is to be expected from the observation that, in the fungi, it is the tips of the hyphae that are concerned in the growth. Translocation of nutrients is an important determinant in the case of fungi, whereas bacteria are submerged unicllular organisms.

Acknowledgments

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A Note on the Correlation Coefficient of the Bivariate Gamma Type Distribution

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1. *Introduction.*

Since the development of the theory of normal correlation by Galton and Dickson (1886) several attempts have been made to describe analytically a distribution of two correlated variables, when both of them follow a low skew variation. In general, very few instances can be cited for studies on bivariate surfaces. Pearson (1925) obtained a fifteen-constant bivariate surface as a double hyper-geometric series, while Kibble (1941) developed the frequency function for the joint distribution of two correlated variables both of which conform to the gamma type. The special distribution derived by Kibble is given by

$$\varphi(x, y) = \frac{1}{\Gamma(p)} \cdot \frac{\varrho^{-(p-1)}}{(1-\varrho^2)} \cdot e^{\frac{-(x+y)}{1-\varrho^2}} (xy)^{p-\frac{1}{2}} \mathrm{I}_{p-1} \left\{ \frac{2\varrho}{1-\varrho^2} (xy)^{\frac{1}{2}} \right\} \quad .. \quad (1)$$

where ϱ and p are parameters. The marginal distributions for this surface follow the gamma type given by

$$f(x) = \frac{1}{\Gamma(p)} \cdot e^{-x} \cdot x^{p-1} \quad .. \quad (2)$$

and have the same parameter p which measures skewness in the population. The other parameter ϱ is the product moment correlation of the related normal distribution.

In this note a functional relationship between ϱ and ϱ^*, the correlation coefficient for the bivariate distribution given by equation (1) has been derived.

2. *Correlation Coefficient* ϱ^*.

Following the usual definition of product-moment correlation coefficient

$$\varrho^* = \frac{\mu_{11}}{\sqrt{\mu_{20}\,\mu_{02}}} = \frac{\mu_{11}' - \mu_{10}'\,\mu_{01}'}{\sqrt{\mu_{20}\,\mu_{02}}} \qquad .. \quad (3)$$

where all the μ's have the usual meaning of bivariate moments. From the marginal distribution given by equation (2) it can be seen that

$$\left.\begin{aligned} \mu_{10}' = \mu_{01}' = p \\ \text{and } \mu_{20} = \mu_{02} = p \end{aligned}\right\} \qquad .. \quad (4)$$

So to evaluate ϱ^* it is necessary to obtain the value of μ_{11}'.

From equation (1), μ_{11}' can be written as

$$\mu_{11}' \int_0^\infty \int_0^\infty \varphi(x, y)\; xy\, dxdy \qquad .. \quad (5)$$

Since $I_n(Z)$, the Bessel function of second kind is given by

$$I_n(z) = \sum_{s=0}^{\infty} \left\{ \frac{(\frac{1}{2}z)^{n+2s}}{s!\,\Gamma(n+s)} \right\} \qquad .. \quad (6)$$

equation (5) can be written as

$$\mu_{11}' = \frac{(1-\varrho^2)^{-1}}{\Gamma(p).\varrho^{p-1}} \int_0^\infty \int_0^\infty e^{\frac{-(x+y)}{1-\varrho^2}} (xy)^{\frac{p+1}{2}} \left[\sum_{s=0}^{\infty} \frac{\left\{ \frac{\varrho}{1-\varrho^2} (xy)^{\frac{1}{2}} \right\}^{p-1+2s}}{s!\,\Gamma((p+s)} \right] dxdy \qquad .. \quad (7)$$

Then, since all the terms within the summation are of the same sign the order of double integration and summation can be reversed. Thus

$$\mu_{11}' = \frac{(1-\varrho^2)^{-p}}{\Gamma(p)} \cdot \sum_{s=0}^{\infty} \left[\frac{\Gamma^2(p+s+1)}{s!\,\Gamma(p+s)} \left(\frac{\varrho}{1-\varrho^2} \right)^{2s} (1-\varrho^2)^{2p+2s+2} \right]$$

$$= \frac{(1-\varrho^2)^{p+2}}{\Gamma(p)} \cdot \sum_{s=0}^{\infty} \frac{\Gamma^2(p+s+1)}{s!\,\Gamma(p+s)} \cdot \varrho^{2s} \qquad .. \quad (8)$$

$$= \frac{(1-\varrho^2)^{p+2}}{\Gamma(p)} \cdot \frac{\Gamma^2(p)}{\Gamma(p-1)} \cdot \sum_{s=0}^{\infty} \left[\frac{(p+s)^2 (p+s-1)^2 \ldots p^2}{s!\,(p+s-1)\ldots(p-1)} \cdot \varrho^{2s} \right]$$

$$\mu_{11}' = (1-\varrho^2)^{p+2} \cdot p^2 \left[1 + \frac{(p+1)^2}{1!\,p} \cdot \right.$$

$$\left. \varrho^2 + \frac{(p+1)^2 (p+2)^2}{2!\,p(p+1)} \varrho^4 + \ldots \right]$$

$$p^2 (1-\varrho^2)^{p+2} F(\overline{p+1},\ \overline{p+1},\ p,\ \varrho^2) \qquad .. \quad (9)$$

in the usual notation for hyper-geometric series, where

$$F(\overline{p+1},\ \overline{p+1},\ p,\ \varrho^2) = 1 + \frac{(p+1)}{1!} \varrho^2 \frac{(p+1)}{p}$$

$$+ \frac{(p+1)\,(p+2)}{2!} \varrho^4 \cdot \frac{(p+2)}{p}$$

$$+ \frac{(p+1)\,(p+2)\,(p+3)}{3!} \cdot \varrho^6 \quad \frac{(p+3)}{p} + \ldots\ldots\infty$$

$$= \left\{ 1 + \frac{(p+1)}{1!} \varrho^2 + \frac{(p+1)\,(p+2)}{2!} \right.$$

$$\left. \varrho^4 + \frac{(p+1)\,(p+2)\,(p+3)}{3!} \varrho^6 + \ldots \infty \right\}$$

$$+\frac{1}{p}\left\{\frac{p+1}{1!}\varrho^2+\frac{(p+1)(p+2)}{2!}2\varrho^4+\frac{(p+1)(p+2)(p+3)}{3!}3\ \varrho^6+\ldots\infty\right\}$$

$$=(1-\varrho^2)^{-p-1}+\frac{p+1}{p}\varrho^2(1-\varrho^2)^{-p-2}$$

$$=(1-\varrho^2)^{-p-2}(1-\varrho^2+\varrho^2+\varrho^2/p)$$

i.e., $F\overline{(p+1,\ p+1,\ p,\ \varrho^2)}=(1-\varrho^2)^{-p-2}(1+\varrho^2/p)$ .. (10)

Substituting the result of (10) in equation (9) we get

$$\mu_{11}'=p^2(1-\varrho^2)^{p+2}\ (1-\varrho^2)^{-p-2}(1+\varrho^2/p)$$

$$=p^2(1+\varrho^2/p) \qquad .. \quad (11)$$

Hence from (3) and (4)

$$\varrho^*=\frac{p^2(1+\varrho^2/p)-p^2}{\sqrt{p}\times p}$$

$$=\varrho^2 \qquad .. \quad (12)$$

Thus the correlation coefficient ϱ^* is seen to the square of the correlation ϱ of the related normal distribution. This simple result can be used for evaluating the correlation for the bivariate distribution given in equation (1).

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Contribution to the Galois Theory

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The Galois theory has been recently simplified by E. Artin [1] and N. Bourbaki [2]. It is the purpose of this note to propose a further simplification. It will be found in the proof of theorem IV.

Let E and E′ be two fields over the same ground field. Thoughout this paper it will be assumed that $E \subset E'$. Let σ denote a K-isomorphism of E into E′. It is well known that $\sigma^{-1}(0) = 0$.

Definition I — Isomorphic mappings of E into E′ will be called "characters" or "projections" according as the multiplicative, or the additive, structure of E is concerned, exclusive of the other.

Definition II — An element $e \in E$ is said to be a fixed point of E under a set of isomorphic mappings $\sigma_1, \sigma_2, \ldots, \sigma_m$ of E into E′ if $\sigma_i(e) = e$ for $i = 1, 2, \ldots, m$.

Theorem I — The fixed points of E under a set of isomorphic mappings of E into E′ forms a subfield of E.

The proof is immediate.

Definition III — Two characters of E in E′ are said to be mutually distinct if there is at least one element of E whose images, under these mappings, are distinct.

Definition IV — Projections $\sigma_1, \sigma_2, \ldots, \sigma_m$ of E into E′ are said to be linearly independent if there is no mapping of the form $\lambda_1\sigma_1 + \lambda_2\sigma_2 + \ldots + \lambda_m\sigma_m$, where the λ_i's, non all zero, are in E′, which has a kernel identical with E.

Theorem II — Mutually distinct characters in E′ are linearly independent projections.

The following proof is due to Artin. The theorem is obviously true in the case of one character. Let it be true in the case of $m - 1$ characters and let $\sigma_1, \sigma_2, \ldots, \sigma_m$ be m mutually distinct cha-

racters. If they are not linearly independent projections, there exists a mapping $\sum_1^m \lambda_i \sigma_i$ of E into E′, where at least two λ_i's are not zero and all the λ_i are in E′, which maps E on 0. There is no loss of generality in supposing that $\lambda_1 \lambda_m \neq 0$. Our assumption is that there is at least one element of E, a say, such that

$$\sigma_1(a) \neq \sigma_m(a) \neq 0.$$

Now $\sum_1^m \lambda_i \sigma_i(ax) = \sum_1^m \lambda_i \sigma_i(a)\, \sigma_i(x)$ whatever $x \varepsilon E$; therefore $\sigma_m(a^{-1}), \sum_1^m \lambda_i \sigma_i(a)\, \sigma_i(x) = 0.$

It follows that $\sum_1^m \lambda_i [\sigma_m(a^{-1})\, \sigma_i(a) - 1] \sigma_i(x) = 0$, $x \varepsilon E$, where $\sigma_m(a^{-1})\, \sigma_1(a) - 1 \neq 0$. But then $m - 1$ mutualy distinct characters are linearly dependent projections and this contradicts our inductive assumption. The theorem holds therefore in the case of m characters, whatever be m.

Theorem III — Let $S : \{\sigma_1, \sigma_2, \ldots, \sigma_n\}$, σ_1, *denoting the identity mapping, be a set of mutually distinct characters of E in E′ and let F be the fixed field of E under S. Then* $E : F \geqslant n$.

Let $E : F = m$. Let $\{e_i\}$ be a linear basis of the vector space E over F. Every linear mapping of this vector space into the vector space E′ over F is uniquely determined by its values on the e_i's. Therefore it is possible to determine m linear mappings $u_1, u_2, \ldots, u_m$ by the conditions $u_i(e_j) = \delta_{ij}$ where δ_{ij}'s are the Kronecker deltas. The u_i's are obviously linearly independent linear mappings of vector space. Let u denote another linear mapping of E onto E′ over F. Let $u(e_i) = b_i$, $i = 1, \ldots, m$. Then $u - \sum_1^m b_i u_i$ is zero on every one of the e_i's and therefore

$$u = \sum_1^m b_i . u_i$$

Now every one of the characters of S belongs to the restriction of the linear mappings of E into E′ to field isomorphisms. It follows that not more than m can be mutually distinct and $n \leqslant m$. This proof is due to N. Bourbaki.

Theorem IV — *Let E be a normal extension of a field K and G: $\{\sigma_1, \sigma_2, \ldots, \sigma_n\}$ a group of K-automorphisms of E, of order n. If F be the fixed field of E under G, E : F = n.*

By Theorem III, $E : F \geqslant n$. Theorem IV will be proved if we show that $E : F \leqslant n$.

Let $a_1, a_2, \ldots, a_{n+1}$ denote any $n+1$ elements of E. The system of linear equations

(1) $\sum_{i=1}^{n+1} \sigma_j(a_i)\, x_i = 0,\ j = 1, 2, \ldots, n,$ has a non trivial solution.

Let it be denoted by (x_i°). If t is a (non zero element of E_1), $(t.x_i^\circ)$ is also a non trivial solution of (1). We may determine t so that one of the elements $t.x_i^\circ$ $t.x_i^\circ = y_i^\circ$ be an element a of E such that Trace

$(\alpha) \neq 0$. There exists such an element, else Trace $(x) = \sum_1^n \sigma_i(x) = 0$.

for every x of E and the σ_i's are not linearly independent projections, thus implying that G is of order less than n. Now the system (1) may be written in the form

$$\sum_{i=1}^{n+1} a_i\, \sigma_j^{-1}(x) = 0,\ j = 1, 2, \ldots, n.$$

It follows that

$$\sum_{i=1}^{n+1} \text{Trace}\ (y_i^\circ)\, a_i = 0.$$

Since all the quantities Trace (y_i°) are not zero, we have a relation of linear dependence between the a_i's. Therefore $E:F \leqslant n$.

Corollary — *If F is the fixed field of E under a finite group G of K-automorphisms, every K-automorphism of E leaving F invariant must belong to G and G is uniquely determined by F.*

The proof is immediate.

Definition V — E is called a Galois extension of K if E is a field extension of K and K is the fixed field of E under the group of all the K-automorphisms of E. This group itself is called the Galois group of E over K.

Theorem V — *E, a normal and finite extension of K, is a Galois extension of K if and only if E is separable over K.*

Sufficiency of the condition: If $E : K = n$ and E is separable over K there exists a primitive element having n distinct conjugates over K. If F be the fixed field of E under all its K-automorphisms, $E : F = n$ and $K \subset F$. It follows that $F : K = 1$ and $F = K$.

Necessity of the condition: If K = F, every element of E not in K has conjugates distinct from itself. Let e be such an element and $e_1, e_2, \ldots, e_m$ its distinct conjugates. Then e is root to the equation $\prod_i^m (x - e_i) = 0$ which is over K and separable.

Theorem VI — If E is a finite, Galois, extension of K:

(1) *there is a one-to-one correspondence between intermediate subfields of E over K and subgroups of G, the Galois group of E over K,*

(2) *there is a one-to-one correspondence between normal intermediate subfields of E over K and normal divisors of G,*

(3) *if B is a normal intermediate subfield of E over K and g the normal divisor of G uniquely determined by this subfield, the Galois group of B over K is isomorphic with G/g,*

(4) *if B is an intermediate subfield of E over K and g the subgroup of G uniquely determined by B, B : K = G : g.*

It is easily seen that (1) is an immediate consequence of the Corollary of Theorem IV and of the fact that E is a Galois extension of any of its intermediate subfields.

To prove (2) we notice that if B is an intermediate subfield of E over K, if B′ denotes one of its conjugates and if g is the group of the B-automorphisms of E, every element of a left coset of G modulo g maps B onto B′ if and only if a single element of the same coset does. If $\sigma(B) = B'$, $\sigma \varepsilon G$, we have $B' = \sigma g(B) = \sigma g \sigma^{-1}(B')$. As $E : B = E : B'$, and g and its conjugate $\sigma g \sigma^{-1}$ have the same order, $\sigma g \sigma^{-1}$ is the Galois group of E over B′. If B is normal: $B = B'$. $g = \sigma g \sigma^{-1}$ and g is a normal divisor of G. Conversely $g = \sigma g \sigma^{-1}$ implies that B is self-conjugate, that is to say, normal.

If g is a normal divisor of G, there is a one-to-one correspondence between the cosets of G modulo g and the K-automorphisms of B. It is not difficult to show that this correspondence is an isomorphism onto. Hence (3).

(4) follows from the fact that $E : K = (E : B)(B : K)$ and that $E : K = G : 1$, $E : B = g : 1$.

Remark — Theorem VI does not hold good for infinite Galois extension.

The application of the Galois theory to the theory of equations is based on the following:

Theorem VII — E is a finite Galois extension of K if and only if E is the splitting field of a polynomial over K which is separable over K.

The proof is not difficult and will be found in [1].

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The 'Blast' Disease of Rice (*Piricularia oryzae* Cav.)

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Piricularia oryzae Cav., a facultative saprophyte, belonging to the group *Hyphomycetes* is the causal organism of the 'blast' disease of rice and has a wide distribution in all rice growing tracts of the world, chiefly Japan, Italy and India. An excellent account of the disease has been provided by Padwick (1950). Even though the genus has been recorded on other hosts such as *Eleusine coracana, Setaria italica, Triticum vulgare, Zingiber officinale* and several grasses, *Piricularia oryzae* Cav., has received the greatest attention from phytopathologists in view of the serious losses to the rice crop, sometimes amounting to 75%. The disease was first recorded on rice in Italy and the pathogen was named *Piricularia oryzae* by Cavara in 1891 and again by Briosi and Cavara in the subsequent year.

Symptomatology:

Although the symptoms are most commonly conspicuous on the leaves, they are also seen on the leaf sheath, rachis, the joints of the culm and even the glume.

The symptoms first appear on the leaves as small, bluish flecks, about 1-3 mm. in diameter which enlarge rapidly into spindles of several cms. in length and 0·5 to 1·0 cm. in width. The spots, at this stage appear pale or dull green at the centre which later turn almost grey with an outer dark brown rim. (Fig. 1a) Not infrequently do several spots coalesce resulting in the withering of the entire leaf. The spots on the leaf sheath are not essentially different from those on the leaf.

On the rachis of maturing inflorescences, however, particularly near the joints of rachillae and the rachis, black spots or rings are observed. Such spots can also be seen on the glumes in the case of heavily infected panicles. The nodes of the culm may be similarly affected (Fig. 1 b & c).

In very severe attacks, the heads emerge prematurely, completely blasted and appearing whitened long before the normal time of ripening. On pulling such panicles which come off freely with the least effort, there can be observed a grey, fluffy mycelium on the severed end of the stem which is also shrivelled (Fig. 2). If the attack is sufficiently early, there is no filling-out of the grain resulting in a chaffy ear-head and should the attack be late, when some grains have already been filled, the panicle breaks at the rotten neck owing to the weight of the grains.

Description of the fungus:

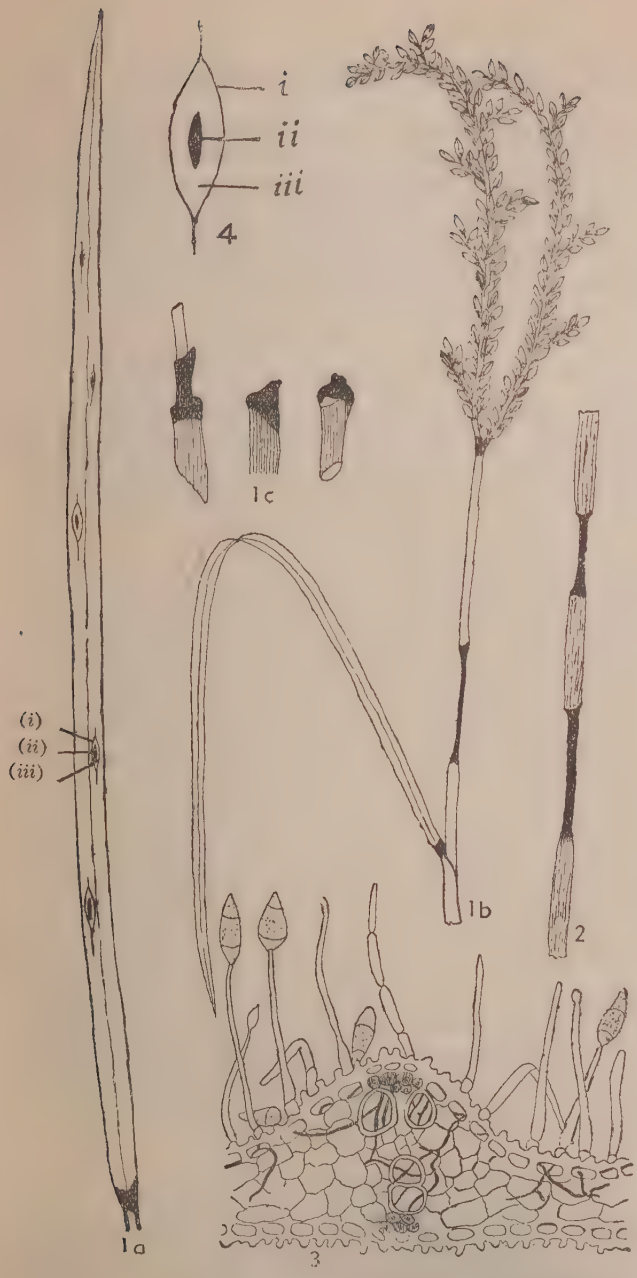
Mycelium in cultures aerial or submerged, hyaline or olivaceous 1·5 to 6 μ in diameter, septate, branched; conidiophores one to many, fasciculate, simple or rarely branched, 2-4 septate, not or slightly constricted at septa; at first monosporic then pleurogenous on sympodium; olivaceous to fuligenous; base swollen, dark coloured becoming light in colour towards apex.

Conidia (Fig. 3) are borne on a conidiophore, acrogenously, being formed on the tips of new branches arising just below the point of origin of the preceding conidium. Conidia are variable in size and shape, terminal, pyriform to obclavate; base rounded, apex narrowed; 2-septate, rarely 1-3 septate, not or slightly constricted at septa, almost hyaline to pale olive, usually 19-23 × 7-9 μ in size with small basal appendage. Conidia germinate from apical or basal cell, and less frequently from middle cell. Germinating hyaline, branched, 3-5 μ in width.

Chlamydospores olivaceous, round or roundish, somewhat thick walled, 5-12 μ diameter, terminal or intermediate, often germinate readily.

Taxonomy:

Based on the morphology, physiology and parasitism, Nisikado (1917, 1927) made an attempt to assign a few isolates of *Piricularia* to their systematic position within the genus. Studying four isolates of *Piricularia* from *Oryza sativa, Eleusine coracana, Setaria italica* and *Digitaria marginata*, Ramakrishnan (1948) did not find any appreciable difference in the morphological characters of the isolates. He also reported that the isolates from *O. sativa, S. italica* and *E. coracana* could not be physiologically distinguished as they behaved similarly on various culture media. An attempt to classify these and a few other isolates on their vitamin hetero-



FIGS. 1–4

1 a—Symptoms on leaves of the rice plant; 1 b—Symptoms on the panicle. Note spore masses (darkened) at the base of the leaf, on the culm, rachids and glumes. The whole ear-head is blasted and the grains shrivelled; 1 c—Nodes blackened by the fungus; 2—Culm drawn out from the surrounding sheath revealing shrivelled areas covered with dark grey hyphae and conidia; 3—T. S. of infected leaf showing conidia, (Diagrammatic, after Cavara); 4.—Single lesion enlarged to show different zones of necroses. (*i*) Venenate zone; (*ii*) Disintegrated zone; (*iii*) Necrotic zone.

trophy similarly proved not successful in this laboratory (Suryanarayanan, 1955). However, cross inoculation studies have proved that *P. oryzae* is distinct inasmuch as it cannot infect any other than its natural host viz., rice, whereas, the isolate from *S. italica* can infect *E. coracana* but not *D. marginata* and *O. sativa*. Similarly, the isolate from *E. coracana* infects also *S. italica* but not *O. sativa* and *D. marginata*. The isolate from *D. marginata*, however, is able to infect *O. sativa* and *E. coracana*. Further, the grasses *Panicum repens* and *Leersia hexandra* are known to serve as alternate hosts for *P. oryzae*. Thus, the taxonomic position within the genus is, as yet, not quite clear and Ramakrishnan (1948) concludes that the various strains of *Piricularia* can at best be considered as physiological races within the genus and species *Piricularia oryzae*.

Life cycle and biology of the fungus:

Even though the fungus can be found within the tissues of the embryo, endosperm, glumes etc., it is not considered seed-borne as hot water treatment of the seeds does not control the disease. Conidia occurring on any part of the diseased rice plant or mycelium within the tissue of the diseased spots are thought to serve as the principal overwintering organs of the fungus. However, recent work in this laboratory has indicated the poor competitive saprophytic ability of the fungus amidst a host of highly antagonistic bacteria and actinomycetes under optimum moisture level of the soil (Appa Rao, unpublished). Nevertheless, under dry conditions, the conidia and mycelium overwinter freely over long periods. The overwintered conidia and those found on alternate hosts like *Panicum repens* growing on the bunds of rice fields readily serve as sources for primary infection. The disease sets in under conditions of high humidity, (above 90%) and with an air temperature ranging from 24-28°C. Such conditions are obtained in the Madras State from November to January and the disease appears in the field during these months.

Mode of infection and spread:

When an air-borne conidium rests on a leaf, where free moisture is at play, it readily germinates. An appressorium is formed, from which a slender penetration hypha is extruded which pierces the epidermal membrane and enters the cell cavity. This penetration of the host may also be effected through the stomata. Inside the cell, a vesicle is formed from which hyphae permeate. After an inter- and intra-cellular invasion of the tissues, conidio-

phores emerge, piercing through the epidermal cells or stoma and produce a fresh crop of the characteristically pear or top shaped conidia. In artificial inoculation studies, it has been found that six days elapse between inoculation, development of symptoms and production of conidia. These conidia when blown by wind serve as inoculum for secondary spread on nodes, culms and neck of the panicles.

Pathological histology:

Three zones in the infected region can be distinguished; the venenate, necrotic and disintegrated. The venenate zone occupies the border of the diseased portion showing a light yellowish stripe that blends into the healthy tissue. This zone is infiltered by toxic substances, now identified as ∝ picolinic acid and piricularine. The necrotic zone is usually a brown, narrow streak along the inner side of the venenate zone. In the venenate zone the pathological changes involved are the discoloration and decrease in the size of the chloroplasts, degeneration of the cell membrane and vacuolar or granular disintegration of the protoplasm. With the spread of the hyphae into the venenate zone, the cell inclusions as well as the cell walls collapse thus forming the disintegrated zone.

Physiology of the Pathogen:

The fungus is unable to grow anaerobically. The minimal, optimal and maximal temperatures for growth of the mycelium has been found to be 8-9°C, 26-28°C, and 36-37°C. respectively.

Recent work in this laboratory on the nutritional physiology of *P. oryzae* indicated that while organic ammonium and amide sources of nitrogen as well as inorganic nitrate nitrogen are assimilated, inorganic ammonium nitrogen is not utilized by the fungus. On the other hand, a Japanese isolate was reported to grow equally well with ammonium sulphate. The problem, however, has now been solved in this laboratory as being due to a rapid fall of pH in the ammonium sulphate medium. If this fall in pH is obviated by suitably buffering the medium with calcium carbonate or if certain organic acids of the Kreb's cycle are present, normal growth of the fungus in such medium is restored (Appa Rao, 1956).

The fungus is able to utilize a variety of carbon sources including starch, cellulose and pectin. Higher alcohols in general are poor sources of carbon.

Growth of the fungus occurs in a pH range of 5-10, the optimum, however, lying between 6 and 7.

The pathogen belongs to the class of vitamin heterotrophs showing a total and absolute deficiency for thiamine and biotin (Leaver *et al.*, 1947, Otani, 1952a, Sadasivan and Subramanian, 1954). The intact thiamine molecule is not required and only the pyrimidine portion of thiamine is needed on a sucrose-nitrate medium. But if sucrose is replaced with glucose or fructose, both the thiazole and pyrimidine fractions are required to complete thiamine synthesis (Suryanarayanan, 1955). A Japanese isolate again differed here in that it needed both the moieties (Tomizoa, 1953).

The essentiality of trace elements for this fungus was for the first time investigated in the University Botany Laboratory, Madras (Appa Rao *et al.*, 1955). The essentiality of Fe, Zn and possibly Cu for growth was indicated in this work. Later, the essentiality of Mn was also proved (Appa Rao, 1956).

Sporulation:

Very exacting conditions seem to govern sporulation of this fungus. Even under natural conditions, at a relative humidity of 93%, conidia are rarely formed on *Piricularia* spots. Henry and Anderson (1948) reported that forced aeration and a temperature of 28°C. was required for the fungus to sporulate on a 1:1 mixture of oats and sorghum. Leaver *et al.* (1947) observed the inability of the fungus to form conidia in liquid cultures. However, work in this laboratory has shown that *P. oryzae* sporulates in liquid medium containing 1% sugar (Appa Rao, 1956). Further it has been found that the trace elements Fe, Zn, Cu and Mn are required for sporulation with Fe and Zn in greater concentrations. Mo appears to inhibit sporulation. Apart from the trace elements, the vitamins thiamine and biotin are required in greater amounts for sporulation than for growth. A pH of 6·5 is most favourable for spore production.

Physiologic races:

Marked differences exist in virulence in different strains of the fungus. Inoue (1939) reported that the ability to decompose cellulose varied considerably with different strains of the fungus and this characteristic was positively associated with pathogenicity.

Work in this laboratory has shown that the virulent strains do not essentially differ from the avirulent strains in their vitamin heterotrophy (Suryanarayanan, 1955).

Disease Resistance:

Kawamura (1940) found that *Oryza cubensis* as well as *Oryza sativa* was susceptible to infection by *P. oryzae*, while *O. latifolia* and *O. minuta* were highly resistant. In the Madras State the strains Co 4, Co 25 and Co 26 have been found to be resistant and ADT 10, the most susceptible to the disease.

Nature of resistance:

The earliest investigations on the nature of disease resistance were mainly confined to anatomical studies and resistance was attributed to the silicification of the epidermis. Akai (1939) stated that seedlings grown under flooded conditions were more resistant by virtue of the greater number of silicated cells in the epidermis. Resistance to 'blast' was also correlated with low number of stomata in the neck region of some Japanese varieties. Thomas (1930) observed that early maturing varieties tended to have a lower percentage of infection and that heavy vegetative growth and the use of nitrogenous fertilizers induce susceptibility to the disease. It was however found that the most resistant variety Co 4 remained unaffected by excessive doses of nitrogen. The effect of excessive application of nitrogen was attributed to the reduction in thickness of the leaves and epidermal cells, increase in the number and size of the stomata, reduction in the sclerenchymatous tissue and an increase in the soft parenchyma. Recent studies in this laboratory and elsewhere have shown that an increase in the soluble organic nitrogen as α amino and amide nitrogen in the plant is associated with susceptibility (Otani, 1952b; Appa Rao, 1956).

In the field heaviest infection occurs on a crop exposed to frequent rains, numerous cloudy days and a high relative humidity. The plants show high resistance at a soil temperature of 28°C., with increase in susceptibility at lower temperatures. There is also evidence that mechanical injury renders the plant more susceptible to infection. It has also been observed that lesions enlarge most rapidly on moderately shaded plants.

According to Hashioka (1947), the prevalence of blast in temperate areas is mainly dependant on a temperature suitable for

the growth of the pathogen. In the tropics, the problem of disease resistance is more complex being dependant upon varietal resistance, relation of temperature to resistance of the host and many other unknown factors. An entirely new bio-chemical approach has been made recently in this laboratory on the problem of disease resistance by examining the nature of exosmosed substances on the resistant and susceptible types. Preliminary investigations have indicated that a number of amino acids and organic acids are present on the leaf surface. The interesting feature of this investigation is that succinic acid while present on the resistant variety is absent on the susceptible. Similarly the amino acid glycine is absent on the resistant type (Subba Rao and Suryanarayanan, 1957). Further work in this direction is in progress and is expected to throw considerable light on the physiology of spore germination at the infection-court and consequently on the mechanism of disease resistance, since, admittedly, the severity of an air-borne disease like 'blast' is indeed a reflection of the number of spores that can successfully germinate on the leaf surface.

Control measures:

Ensuring adequate water supply in the nurseries, observing field sanitation like burning diseased straw and stubbles, keeping the bunds clean off weeds like *Panicum repens* etc., and judicious manuring are some of the prophylactic measures advocated. Other methods of control include the use of copper sprays like Bordeaux mixture and 'Perenox' in nurseries, on transplanted crop and just before ear emergence. The most efficient method of obviating the disease has however been found to be the growing of resistant varieties. The varieties Co 4, Co 25 and Co 26 have been promising in this regard in the Madras State.

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